Effect of pomegranate (Punica granutum) and rosemary (Rosmarinus officinalis L.) extracts on shelf-life for chilled Greenland halibut (Reinhardtius hippoglossoides) fillets in modified atmosphere packaging at 2 ºC

Ünalan, U.; Dalgaard, Paw; Korel, F.

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PREFACE

It is our pleasure to introduce you **The International Food Congress** entitled **"Novel Approaches in Food Industry"** which will be held in Çeşme, Izmir, TURKEY. The congress will take place on 26-29 May, 2011 and include a variety of hot topics such as novel food products and technologies, thermal and non-thermal food processing technologies, applications of nanotechnology in food processing, innovations in food science and technology. This congress will highlight the most important areas of recent Research & Development in Food Science and Technology as well as explore relevant and interesting topics for the future. The congress will also provide accurate and updated scientific information and trends for the discipline of food science and technology. 400 leading scientists from all over 40 countries will contribute to the congress as oral or poster presentations.

This congress will provide a forum for the exchange of ideas and authoritative views by leading scientists, as well as business leaders and investors in the food industry. **More than 32 leading food industry companies became sponsor or supporting organization to our congress**. Outstanding keynote speakers and well-known leading scientists and experts from around the world will be sharing their knowledge with us. Company executives, as well as speakers from universities, research centers and governmental institutions will discuss scientific and technical developments in detail.

We would like to thank all contributors including authors of oral and poster presentations and our sponsors for contributing to the success of this congress.

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INVITED SPEAKER

Advances in nanotechnology for agriculture and food

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Introduction

The applications of nanotechnology in food and agriculture are new as compared to their use in medicine and pharmaceuticals. Advances in technologies such as DNA microarrays, micro-electromechanical systems, microfluidics and nanocomposites are leading to applications focused on food technologies that are unique and empowering. The use of nanoencapsulation, nanotubes, nanoemulsions, nanoparticles and nanosensors enabled food scientists to successfully deliver nutrients and nutraceuticals and detect pathogens and contaminants in a more efficient way, develop packaging materials that are green and have good mechanical and barrier properties and develop miniaturized analytical tools for various applications, and develop imaging tools to characterize structure and chemistry better than before.

Nonoemulsions and nanoencapsulations

Controlled release of active compounds can be achieved by nanoencapsulation of nanospheres including micelle, liposome, nanoemulsion and biopolymeric nanoparticles. Micellar encapsulation was used to encapsulate beta carotene with a chitosan micelle[1,2]. Nanosized particles resistant to a physiological environment prolonged the lifespan of active compounds in bloodstream resulting in an increase of bioavailability. Self-assembly of food proteins at the nano-scale can be useful for binding components such as vitamins or enzymes, to protect encapsulated nutraceuticals and mask undesirable flavor or aroma compounds. Nanoemulsion and encapsulation techniques were used to synthesize a liposome based nanosensor with encapsulated enzymes and a fluorescent indicator[3].

Nanomaterials for imaging

Quantum dots (QDs), have been used in imaging and have been deployed as markers for biological processes. QD- immunolabelling has proved to be effective for detection of several pathogenic bacteria beneficial in food safety considerations. The used of QDs to bind protein molecules for probing protein distribution in bread has been reported [4]. Carbon nanotubes (CNTs) are good carriers for delivery of biomolecules because of their easy penetration to cell membranes inducing apoptosis or cell death [5]. Nanofiber entrapped with QDs improved the enzyme immobilization that can be useful in bioreactor [6]. Polymer nanoclay nanocomposite technology has been proven to improve the mechanical, barrier and thermal properties of several synthetic polymers and biopolymers for packaging applications. The enhancement is a result of well- dispersed 1 nm single layers in polymer matrices. The incorporation of nanoclay into zein film has shown that adequate preparation methods together with the right chemical affinity between zein and nanoclay would result in physical properties improvement [7].

Micro and nanofluidics

Microfluidic devices are widely used in the design of both bio-analytical and diagnostic micro-devices. Miniaturized chips have several advantages including consuming small amount sample and reagents, reducing the requirement of laboratory spaces, labor and expertise. The applications of microfluidic devices include mixing and creating nanoemulsions, detection of contaminants, toxicants and pathogens in

foods [8, 9]. Recently, the demands of environmentally friendly polymers such as natural derived polymers has significantly increased Zein's properties have led to successful design and fabrication of zein microfluidic devices for the first time[10].

Summary

Nanotechnology is beginning to have a noticeable impact on food technology. Nanoemulsion and nanoencapsulation technologies are good tools for producing high-bioavailability-encapsulated bioactive compounds. QDs assist chemical and biological materialdetection by their powerful imaging ability. The use of nanoparticles such as carbon nanotubes and nanoclays result in many useful functionalities. Miniaturized microfluidic systems enable portable and disposable microchips for rapid diagnosis.

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INVITED SPEAKER

Ozone use for sanitization or decontamination of food, water and environment

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Ozone, a triatomic oxygen molecule, has a high oxidizing power and potent antimicrobial properties. Most bacteria, fungi, viruses and protozoa are sensitive to ozone treatment. Ozone has numerous applications in its gaseous or aqueous state. These include food, water, medical and environmental applications. For example, ozone is widely used in the treatment of municipal and bottled water, as a safer alternative to chlorine. Potential food applications include sanitization of fresh produce and whole shell eggs, and decontamination of nuts and spices. Ozone is also capable of reducing pesticide residues and mycotoxins on some food products. Methods to use ozone to sterilize medical instruments are being developed. Ozone-based washers are available for cleaning and sanitizing walls and floors of food processing facilities.

At the Ohio State University, we have an active ozone research program. Under this program, a method to produce *Salmonella*-free eggs has been patented and is currently in the implementation phase. Recently, a method was developed to eliminate the enterohemorrhagic *Escherichia coli* on delicate fresh produce such as spinach and strawberries. However, there are challenges that limit ozone use in some applications. Some of these challenges are due to the nature of ozone itself; e.g., its short half-life. Others are related to the equipment that generates the gas. Well-thought designs are needed for treatment chambers where gaseous or aqueous ozone comes in contact with the treated product. Control of ozone in work environment is essential for the safety of equipment operators and other workers in the facility. These applications and challenges will be presented in detail during the meeting.

INVITED SPEAKER

May food process engineering research and innovation help in facing society problems in a fast changing world?

Heike P. Schuchmann

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Our civilisation is changing faster than ever before. Information and knowledge double every 5-10 years. The global human population is still growing. Three billion people lived on earth in the year 1960, today 6.5 billion are living on our planet, and in the year 2050 the population will be 9 billion, most of them hoping for a better standard of living than today's. As a result of the growing population, the consumption of resources increases faster and faster. Most of these resources, like fossil fuels, are not renewable and moreover, the use of some may cause climate change. Ensuring high quantity and quality food for an extremely growing world population is one of the challenges food engineers may contribute to. In parallel, society has to face new problems arising from the consumption of unbalanced food in unhealthy quantities especially in highly developed (industrialized) countries. Health-care costs explode and quality of life decreases significantly for those facing cancer or cardiovascular decease arising from the new lifestyle. Solving these problems also demand close cooperation between medical, sociological, physiological and also food engineering specialists.

Facing these challenges, food process engineering offers some promising developments. Intensified research in the following areas may induce substantial improvements and technical innovations:

1. Development of adjusted processes and food products by employing the knowledge established in basic chemical engineering using modern tools such as computeraided simulation, material sciences, novel measuring devices (e.g. magnetic resonance tomography), nano technology, etc. Research in these fields will lead to a better understanding of the relationship between micro- and submicro-structures and functional properties such as bioavailability or bioactivity of food compounds and enable a target product design.
2. Improving the process efficiency, saving of energy and water, reduction of waste and environmental pollution to reach high quality food at the lowest cost and improved sustainability
3. Ensure food safety, for example by improving hygienic design, by providing appropriate packaging and by developing and verifying better models to ensure food safety using quantitative microbiology and new mathematical tools to improve microbial risk assessment (as e.g. the expanded "Fermi solution").
4. Improved product quality control by computer-aided automation tools, advanced monitoring and control systems and flexible manufacturing systems to manage food processes even with many complex interacting parameters involved.

Based on research during the last decades, promising novel processes such as high pressure processing, pulsed electric field treatment or cold plasma treatment to decontaminate food and packaging surfaces start to become globally marketable developments. Functional foods are another new development of increasing interest since the 1990es driving the application of new technologies, such as micro- and nanostructuring foods and encapsulation techniques. Even if nano-technology itself will not be the key in product development, it will drive innovations in understanding process and product-structure-relationships. If these novel processes will someday become true innovations like heat pasteurisation, cooling and freezing of food, is unknown at present.

True innovations are big steps in food process engineering and rare events. Commonly, innovations in food engineering are mostly renovations or improvements of existing processes, which means taking smaller steps.

In order to remain competitive and successful, food industry needs continuous renovations which demand continual research and development. Beside R&D in industry, research in academia and similar institutions is necessary not only to broaden the basic knowledge and to create new ideas and results, but also to qualify and supervise students and young open-minded researchers. Young food engineers in cooperation with natural scientists will become the driving force for the future of food process engineering. Interdisciplinarity will be a key in solving the new society problems. Thus excellent education of adequate young people is the major challenge for the future of our discipline in a changing world with fast growing knowledge in science and technology.

With the help of some recent research results, the general statements mentioned before will be elucidated and discussed.

INVITED SPEAKER

Understanding gastric digestion to develop foods for health

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The quest to manufacture foods for healthy benefits is an underpinning goal of the modern food industry. Recent evidence indicates that the rate of food structure break down during gastric digestion has a significantly affect on the rate of nutrient uptake in the gastrointestinal (GI) tract. Therefore any capability to predict disintegration of food in the stomach is important for developing new food products with novel health benefits. Understanding the post-ingestion food behavior and the knowledge of the availability of nutrients and their uptake kinetics can guide food processors to select appropriate ingredients and processing conditions at the time of manufacture. Results obtained from recent research on the kinetics of disintegration of different types of foods as affected by realistic physiological conditions and food material properties will be discussed. Findings from this research provide an improved understanding of the interaction of the food matrix and active ingredients during gastric digestion. These results provide new information for the food processing industry to develop structured foods for healthful benefits.

INVITED SPEAKER

Environmental impacts of the energy utilization in the food industry

Mustafa Özilgen

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European food industry had 913 billion Euro /year of turnover in 2006, it was the largest manufacturing sector in EU, and created 13.4 % of all the revenues. It provided jobs for 4.3 million people (13.5% of the total employment) and had 16 billion Euro of R&D budget. Food industry is among the major consumers of energy. Almost all the industrial sectors, including the food industry, are trying to improve their energy efficiency, and reduce their energy consumption intensity. Investment and research for promoting the use of renewable energy sources are vulnerable the detrimental effects of the fluctuation of the fossil fuel prices. When the fossil fuel prices go up research an investment for the utilization of the alternative energy sources increases, and when the fossil fuel prices go down most of the investment is lost, the facilities are closed. Availability of some of the conventional food ingredients are affected by the fossil fuel prices. They are employed for biodiesel production when the fossil fuel prices are high; they become food ingredients again when the fossil fuel prices change the other way.

Turkey has population of about 70 million. Turkish economy ranks 17th in the world and the modern Turkish food industry have been integrated to the EU by all means. Inefficient utilization and choosing expensive resources for production makes energy the most expensive input of the industry and reduces the ability of the Turkish companies to compete with the others.

In the lecture, energy utilization in the food industry will be reviewed by referring to Professor Özilgen's ongoing research and its impact on the environment will be discussed by pointing the means to make things better.

European Union and Turkish Legislation on flavourings

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In late 2008, the European Union adopted Food Improvement Agent Package (FIAP), which consists of four new regulations providing a common authorisation procedure for food additives, food enzymes and food flavourings, and individual regulations on enzymes, additives and flavourings.

Regulation (EC) No.1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods came into force on 20 January 2009 as a part of FIAP, with most of the measures applying from 20 January 2010. This regulation does not apply to smoke flavourings, which are already covered by Regulation (EC) No. 2065/2003.

Turkish Food Codex Regulation, which was published in 1997 and last amended in 2010, sets out provisions on flavourings. There is no separate regulation for smoke flavourings.

This presentation covers the European Union & Turkish legislation on flavourings and answers the below mentioned questions:

- What are the essential elements of Regulation (EC) No.1334/2008 and what has changed compared with the previous legal situation?
- What are the specific requirements for use of the term "natural"?
- What are “Biologically Active Principles”?
- For which flavourings and source materials is an evaluation and approval required according to Regulation (EC) No.1334/2008?
- Which flavouring substances are allowed to be used?
- How smoke flavourings are regulated?
- What are the differences and similarities between European Union and Turkish legislation on flavourings?

The sustainability standards and applications in the food industry

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Abstract

Nowadays climate change and shrinking the natural resources are the urgent and serious global problems. Today's consumers and shareholders expect organizations to meet standards of social, environmental and economic performance. Sustainability is accepted as the only concept to overcome these problems. It becomes day by day the key driver for innovation. In near future, the companies shall make sustainability a goal to achieve competitive advantages and that is only possible by redesigning the business models as well as process and product technologies. In coming 40 years there will be living 30% more people in the world. To survive and "living within the limits of the planet" is only possible with sustain of the available natural sources and without further harm to biodiversity, climate and other ecosystems.

Introduction

United Nations has defined the sustainable development as meeting the needs of the present without compromising the ability of future generations to meet their own needs. Sustainability isn't burden on bottom lines, whereas becomming enviroment friendly can lower the costs and increase the revenues. Today's shareholders expect organizations to meet standards of social, enviromental and economic performance. Food manufacturing is one of the most important subject in the sustainability. Starting from agriculture untill the consumers and waste threatment in each process step trying to be the limits is the key factor for continue existence and civilization. For that purpose, some sustainable standards had been developed for helping the food manufacturer to keep within these limits.

Mainly sustainable standards are grouped into tree sections, those are social, enviromental and economic sustainability (Figure 1).

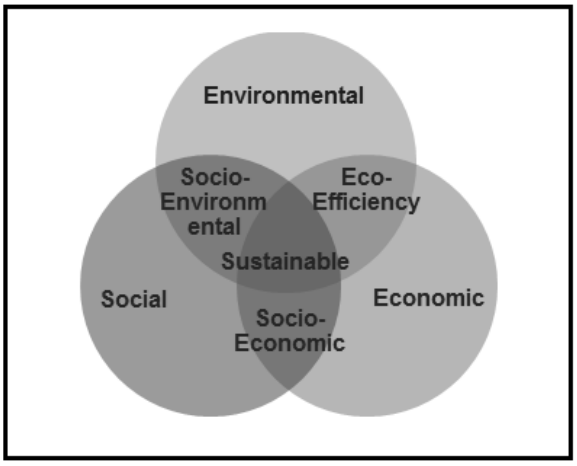

Figure 1. The sustainability triple bottom line

Environmental sustainability focuses on preserving a viable environment and maintaining a sufficient natural capital: Resource, Climate change, Biodiversity. Greenhouse gas emissions, carbon and water footprint, organic foods, biodiesels, energy saving, biodiversity and green building standards will be explained in this group. Social sustainability promotes Human Rights, Diversity, Health and Safety and

solidarity at community and individual level. Economic sustainability aims at developing and maintaining sufficient economic revenue by preventing risks and guaranteeing customer satisfaction. Accountability, corporate governance, economic performance, financial objectives, risk management and resource care are under this scope.

Materials and Methods

There are several types of assessments to ensure the implementation and impact of sustainability practices within the supply chain. Implementation of sustainable development lead to an increasing interaction and interdependency of sustainability pillars. The first step to commit for sustainability and to demonstrate management of processes, the well known solutions are enviroment, quality, health and safety management system certifications. In those management standards uses the Plan-Do-Check and Act approach.

- ► **PLAN** the deployment of a sustainability strategy, to address the different challenges of your business. Corporate commitment is stated in a ***Sustainable Development Policy.***
- ► **DO** things right. Design, Purchase and/or Implement sustainable solutions.
- ► **CHECK** the implementation and fulfilment of your objectives, outline current benefits of your sustainability actions.
- ► **ACT** for improvement of your sustainability practices.

The enviromental schemes are external quality. In that area, the main standard is ISO 14001, this standard aims at limiting, controlling and reducing the enviromental impact. Nowadays a lot of food companies are certified under this scheme. The first reason is related to the interests of the brands and food companies and the second is related to the societal interests in enviromental improvements in food chain.

Another important area is the climate change and reducing the green house gas emisson in the life cycle of a product. This is one of the main action to combat with global climate change. The theory of applying these standards and related certifaciton is first measuring the emisisons, second decreasing the amount and third offsetting the remaining emisisions. Nowadays mainly the retailers such as Tesco, Walmart and the investor groups such CDP are has started to request these standards from the food manufacturers. In this area there are several standards for qualifying and quantifying the GHG emissions. The main schemes are ISO 14064, PAS 2050, ISO 14044 LCA, ISO 14046 Water Footprint, ISO 14067 Carbon Footprint.

'Carbon footprint' is a term used to describe the amount of greenhouse gas (GHG) emissions caused by a particular activity or entity, and thus a way for organisations and individuals to assess their contribution to climate change. Understanding these emissions, and where they come from, is necessary in order to reduce them. Corporate Carbon footprint is measuring the carbon footprints due to the companies own emissions.

ISO 14064 series standards are mainly carbon inventory standards for the coorporations. It is also called corporate carbon footprint. This standard has three part.

- ISO 14064-1 defines the principles and requirements for designing, developing, managing and reporting organization or company-level GHG inventories. It includes requirements for determining GHG emission boundaries, quantifying an organization's GHG emissions and removals and identifying specific company actions or activities aimed at improving GHG management.
- ISO 14064-2 focuses on GHG projects specifically designed to reduce GHG emissions or increase GHG removals. It includes principles and requirements for determining the project baseline and for monitoring, quantifying and reporting the project performance relative to the baseline.

- ISO 14064-3 describes the actual validation or verification process. It specifies requirements for components such as verification planning, assessment procedures and the evaluation of GHG assertions. ISO 14064-3, therefore, can be used by organizations or independent third parties to validate or verify GHG reports and claims.

Nowadays the companies are increasingly concerned with emissions across their entire supply chain. The product carbon footprint refers to the GHG emissions of a product across its life cycle.

PAS 2050 is a publicly available specification for assessing product life cycle GHG emissions, prepared by BSI British Standards and co-sponsored by the Carbon Trust and the Department for Environment, Food and Rural Affairs (Defra). PAS 2050 is an independent standard, developed with significant input from international stakeholders and experts across academia, business, government and non-governmental organisations (NGOs) through two formal consultations and multiple technical working groups.

The International Organization for Standardization (ISO) is working on a new standard for "*Carbon Footprints of Products*" for the quantification and communication of GHG emissions associated with goods and services. The standard builds largely on the existing ISO standards for life cycle assessments (ISO 14040/44) and environmental labels and declarations (ISO 14025) and is planned for final publication in 2012. In comparison to the existing LCA standards it contains further provisions for the uniform quantification of GHG emissions. The standard currently is in *Committee Draft* status, implying that the main provisions have been set and commenting is now taking place on a per country basis.

Also there are some initiatives which aims securing the enviromental performance of agricultural products. **GLOBALGAP** is an internationally recognized set of farm standards dedicated to Good Agricultural Practices (GAP). Through certification, producers demonstrate their adherence to GLOBALGAP standards. For consumers and retailers, the GLOBALGAP certificate is reassurance that food reaches accepted levels of safety and quality, and has been produced sustainably, respecting the health, safety and welfare of workers, the environment, and in consideration of animal welfare issues.

Responsible fishing aims at ensuring responsible fishing practices. The solutions are centered around six areas of responsibility:

- Marine resources: improving selectivity and stock control, reducing waste of by-catch, maintaining the ecosystem
- Environment: saving energy, limiting pollution, sea spillages and loss of gear
- Food safety: ensuring seafood product safety in board and in the seafood chain
- Crew: improving workplace safety and well-being, increasing awareness of social regulations
- Quality: guaranteeing product and process quality, and traceability/origin for clear responsibility at each step
- Local development and equitable share

Responsible Aquaculture supplies 50% of all aquatic products for human consumption worldwide. Aquaculture has seen a steady growth over the past two decades and has strongly contributed to the worldwide decrease in fishing. Sustainability concerns of aquaculture products have pushed producers, processors, wholesalers and distributors to demonstrate the health and well-being of the animals, food safety, quality of feed, environmental protection and social responsibility. To this end, they seek to ensure the sustainability of their business by proving their responsible contribution to the aquaculture industry.
Organic certification is an internationally recognized set of standards aimed at proving that agricultural and food products have been produced without the use of synthetic chemical products, while using methods which respect the environment and animal welfare. Organic requirements involve a set of

production specifications for growing, storage, processing, packaging and shipping. All organic assessment and qualification references require control and certification by an accredited independant body.

AIM Progress Supplier Assessment, 24 Fast Moving Consumer Goods Manufacturers (FMCG) such as Unilever, Coca-Cola, Pepsico, Ferrero, Kraft Foods gathered into a global forum called AIM Progress (Association des Industries de Marque–European Brands Association/ PROGramme for RESponsible Sourcing), which seeks to promote responsible sourcing in order to improve efficiency and effectiveness of the supplier's assessments and to reduce the duplication of the evaluation. Suppliers are therefore encouraged to share their assessment reports directly with these companies upon request or upload them via the Supplier Ethical Data Exchange (SEDEX) platform, which provides a secure online data exchange between suppliers and customers. AIM progress program is based on four pillars (Figure 2).

Figure 2. AIM progress program pillars

Biofuels and biomass have been heavily promoted to be part of the solution to mitigate climate change, as well as anticipate the scarcity of fossil fuels in the future. Governments in Europe, the USA and other countries such as Brazil are setting high targets for the use of biofuels in the coming years through fiscal incentives to promote these sources of energy. This results in many market opportunities for many actors within the biofuels and biomass value chains, from biomass producers (agriculture and forestry) to biofuels producers and distributors. Nevertheless incentives for biofuels and biomass can bear risks and limits that need to be controlled and managed over the supply chain for them to remain sustainable and renewable sources of energy. Typical threats to be managed are negative effect of price of food, deforestation, illegal work conditions or control on greenhouse gas emissions, for which governments, NGOs and public opinion need to be assured.

The European Union's Directive 2009/28/EC on energy from renewable sources, has set specific requirements for the verification of biofuels and ensure their sustainability so that they can be considered by member states to fulfil their objectives. To follow this new legislation the sustainability of the biofuels will need to be verified and guaranteed to be distributed on the European market.

The sustainability of the biomass used for the production of the different types of biofuels can be evaluated against requirements included in recognised voluntary certification schemes and through independent audits. Independent verification needs to cover all the actors throughout the supply chain, from the biomass producer to the final biofuels distributor.

The other bottom lines are the social and economic sustainability. In social responsibility main standard is OHSAS 18001 which aims at assessing and reducing the safety risks in work enviroments. Organizations around the world, and their stakeholders, are becoming increasingly aware of the need for and benefits of socially responsible behaviour. The objective of social responsibility is to contribute to sustainable development. The current global business environment is motivating the food manufacturers and the organizations to consider the full social and ethical impacts of their corporate activities and policies. Those companies who are able to prove a responsible approach to broader social and ethical issues will gain a vital competitive edge and inspire the confidence of stakeholders such as client, investors, local community and consumers. Social concerns like child labour, forced labour and discrimination require companies to not only consider their own direct sphere of influence but also their entire supply chain. The

concept and value of employing an independent, third party to monitor social responsibility is becoming increasingly important.

SA8000 is the first auditable standard in this field. SA8000 standard involves the development and auditing of management systems that promote socially acceptable working practices bringing benefits to the complete supply chain. ISO 26000 is a guide on social responsibility.

Results and Conclusion

In conclusion, we would say that today our society is on the dangerous way of unsustainable living. Actually, we have what is needed to be sustainable, those are scientific knowledge, instant communications, emerging technologies and financial assests. The problem is most of the countries are suffering from inadequate corporate governance and public policies. It would be not so easy and also might be late if we wait the macro solutions driven by the goverments and unions. For reaching the optimum solutions all societies and individuals should react together and change the life style to a sustainable way as soon as possible. But these activites should be managed in a control to gain the most success, it could only be done by the most widely accepted sustainability standards.

Food industry is one of the very important player in the sustainability, There is a need for adopting the sustainability standards that records and exchange information up and downstream in the food chain and allows both primary producers and food industry to continuously benchmark their performance and the retail sector to assure their customers that the products are enviromentally sustainable.

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Flavor dynamics of foods: a case study

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Communication and transfer of precise ideas are so important when dealing with matters as subjective and ambiguous as food flavors and flavorings. A workable language for describing food flavors is the one which the food developer and consumer can use and understand. Food systems are dynamic and flavors are susceptible creatures to all the variables that make food product development a challenging endeavor. The purpose of this study is to (i)better describe the complexities of a flavor profile, and (ii) study a food system , e.g. bakery and/or dairy, that can be more objectively formulated. Therefore, with this knowledge we should be better equipped to describe and use all those misunderstood as mystical ingredients.

A new comprehensive GC-MS method for identification of the aroma profile of traditional Greek distillate ouzo

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Abstract

A chromatographic method was developed for analysis of the aromatic substances of Greek traditional distillate called ouzo. The method was based on the use of a new very long (100 m) capillary chromatographic column which was adjusted on the Agilent GC-MS apparatus and the suitable chromatographic parameters where established after intensive trials and optimization. Thirteen samples of ouzo were analyzed in triplicate by using the developed analytical protocol and the aromatic profile of them was recorded qualitatively and quantitatively for the main aromatic compounds existent in the distillate. A large variety of over fifty aromatic compounds were identified by using the NIST MS-library and most of these compounds found to have 5C and 6C aromatic rings. Furthermore, the main aromatic constituent was found to be trans - anethole while the toxic substances of methanol and cis - anethole were absent. These findings are considered very positive for the quality of the distillate as this appears to have a strong aromatic character without the presence of substance that can harm consumer health. This work claims, for the first time, a comprehensive qualitative analysis for the ouzo distillate and the development of a prototype and general use GC-MS method for determining aromatic substances in alcohol distillates.

Key words: Ouzo, GC-MS analysis, aromatics, distillate

Introduction

Ouzo is a distilled alcoholic spirit which is vastly produced all over Greece and its consumption is part of the people's culture [1]. A series of aromatic materials had been used to improve the flavor of the spirit like anise seed, star anise seed, cinnamon, salvia, mastic etc. In most cases the alcohol content of ouzo varies in the range of 40-43 ˚v/v. Its production is based on the co-distillation of a 50% v/v water/alcohol solution after addition of aromatic materials in it at a percentage of about 3-5% w/v.

However, and despite the importance of its consumption a comprehensive qualitative and quantitative analysis of the spirit concerning the content of either aromatic or potentially hazardous material is lacking from the international scientific literature. Some trial to contribute on this subject were recorded [1,2,3,4,5] but because either a limited number of ouzo samples were analyzed in them or the use of GC methods with confined capacity to provide a deep insight of the qualitative aromatic profile (mainly due to the use of short length GC columns) prohibited the illustration of the full picture of the profile of ouzo volatiles reducing thus the potential for the data to be effectively used by medical teams in order to disclose the presence potentially hazardous substances in the spirit.

In the present work a new approach was used in order to better analyze the qualitative volatile profile of ouzo. Particularly, more samples were analyzed qualitatively by using GC-MS analysis employing a large (100 m in length) capillary column and a quantitative determination was performed using suitable GC-grade standards of the pure volatiles to produce the calibration equations for the main aromatics included in ouzo spirit. This method can be used without any modification for the determination of the volatile profile of a series of distillates like the Turkish raki or the Syrian arak assisting in achieving a better qualitative identification of minor aroma constituents that might have a negative effect on human health despite their low concentration in the above mentioned spirits.

Materials and methods

Chromatographic hardware

An Agilent GC-MS system type was used which was equipped with a 100 m long capillary GC-column type J&W HP88 112-88 A7 100m x250μm x 0.25μm, silica type.

Chromatographic method

The developed chromatographic method was based on the following parameters:
Injection mode : Split with ratio: 100:1
Injection size : 2.5 μl Sample Dilution : none
Oven Program : 50 degrees C foe 5 min, then at 5 ˚C/min to 130 ˚C and finally at 3 ˚C/min up to 250 ˚C
Carrier gas: helium-GC-grade
Heater Temperature : 300 ˚C
MS parameters : LowMass:: 50.0 High Mass: 450.0 MS-Source temp: 230 ˚C MS quad temp.: 150 ˚C.

Ouzo Samples & Chromatographic standards of methanol, estragole & trans-anethole

A set of 13 samples of ouzo obtained from different suppliers around Greece was used for the GC-MS analysis. Each one of these samples was analyzed in triplicate and the average figures were used for quantitative determinations of the main aromatics: methanol, estragole, trans-anethole. The calibration curves used for the quantitative determination of the three main aromatics were constructed by using dilutions of chromatography grade standards obtained by RIGAS LABS S.A-Chemical Supplier. – Thessaloniki Greece.

Results and discussion

a) The qualitative profile of ouzo

A total of 45 volatile substances were identified by using the developed GC-MS analytical method. The total number substances result a significant improvement compared with the ones obtained in previous works. These substances are presented in the Table 1 which follows along with their proposed chemical structures. Most of them are volatile chemicals with 5 C and 6 C rings and most of them well known chemical.

The panorama of these substances can be used by medical research groups in order to identify potential hazardous materials contained in ouzo while the method can extend its application on other distilled spirits like the Turkish raki or Syrian arak. The large number of volatiles which was managed to be obtained is a proof that the method of the present work is superior of others developed in the past.

From the study of the chromatographic charts two major aromatic substance identified in the samples trans-anethole while estragole was also present. Another important finding was that toxic cis-anethole and methanol were not present in all ouzo samples and a variety of volatile chemical substances of different classes was contributing in the aromatic profile of ouzo including esters, alcohols, aldehydes, cetones, cyclic compounds, organic acids and amino acids.

b) Trans-anethole content of ouzo samples

The trans-anethole content of ouzo samples are presented expressed in ppm and in grams/100 litres of pure ethanol in Figure 1,2 respectively.

Table 1.The aromatic profile of Greek ouzo

No	Identified Substance name (NIST database)	Elution time (min)
1	Acetaldehyde, hydroxyl-	**21.040**
2	2- Propanone,1- hydroxyl-	**22.361**
3	Benzene,1- methoxy-4-(1-propenyl-)	**32.667**
4	2-Furancarboxaldehyde,5-hydroxy-	**51.047**
5	2-Hydroxy-gamma-butyrolactane	**51.372**
6	Alanine	**6.196**
7	Acentic acid,oxo-	**6.623**
8	1- Adamantanemethylamine,a	**9.301**
9	Acetic acid (aminocarbonyl-)	**24.338**
10	4-Pyridinol,acetate (ester)	**26.538**
11	Formic acid,ethenylester	**29.720**
12	Benzaldehyde,4-methoxy	**43.379**
13	4H- Cyclopropa(5,6)benz(1,2)	**56.743**
14	Methyl Alcohol	**8.666**
15	Estragole	**32.702**
16	Methanone, (3,4-diethoxyphenyl-)	**46.862**
17	2-Furanmethanol	**29.714**
18	2-Propenoic acid,ethenylester	**38.922**
19	1,6,3,4-Dianhydro-2-deoxy-a-c	**50.076**
20	Acetic acid,oxo-,methylester	**23.753**
21	Acetic acid (acetyloxy)	**24.378**
22	1-Formyl-3-methylaziridine-2-	**24.627**
23	Furfural	**26.548**
24	Propanoic acid,2-oxo-	**27.900**
25	Pyrazole-4-carboxaldehyde	**30.415**
26	4(1H)-Pyrimidinone	**30.903**
27	Benzene, (2- methoxy-2-propenyl-)	**32.722**
28	o-Methylisourea hydrogen sulphate	**24.388**
29	Methylenecyclopropanecart	**29.724**
30	Pyrazole-4-carboxaldehyde	**30.390**
31	4H-Pyran-4-one,2,3-dihydro-3	**45.599**
32	Butanenitrile,2,3-dioxo-,dioxil	**22.325**
33	Pilocarpine	**26.482**
34	Cyclopentanepropanoic acid	**51.377**
35	1-n-Butoxy-2,3-dimethyldiazin	**17.249**
36	Acetic acid,hydrazide	**22.325**
37	Pyrimidine-2,4(1H,3H)-dion,5	**27.885**
38	1H- Pyrolizine-7-methanol,2,3	**41.046**
39	‘2H-Tetrazole,2-methyl-	**42.261**
40	1,3,5-Pentathionethione,1-(4-methyl)	**52.134**
41	3-Amino-2-oxazolidinone	**27.900**
42	1-(3-Methylbutyl)-2,3,4,5 tetra	**30.253**
43	Acetonitrile,hydroxyl	**32.677**
44	Propanoic acid,2-(aminooxy)	**24.739**
45	Butahedioic acid,2,3-bis(acetate)	**27.844**

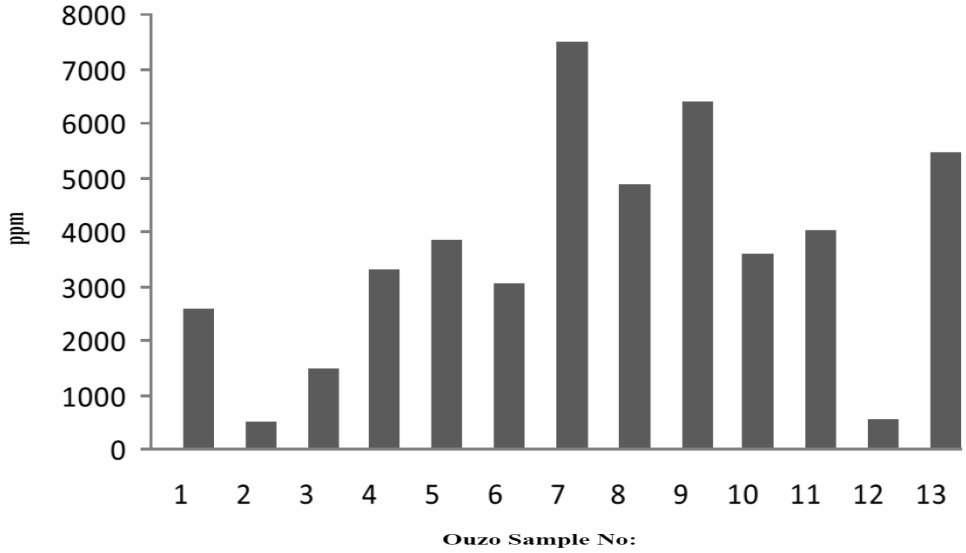

Figure 1. The concentration (ppm) of trans-anethole in various ouzo samples

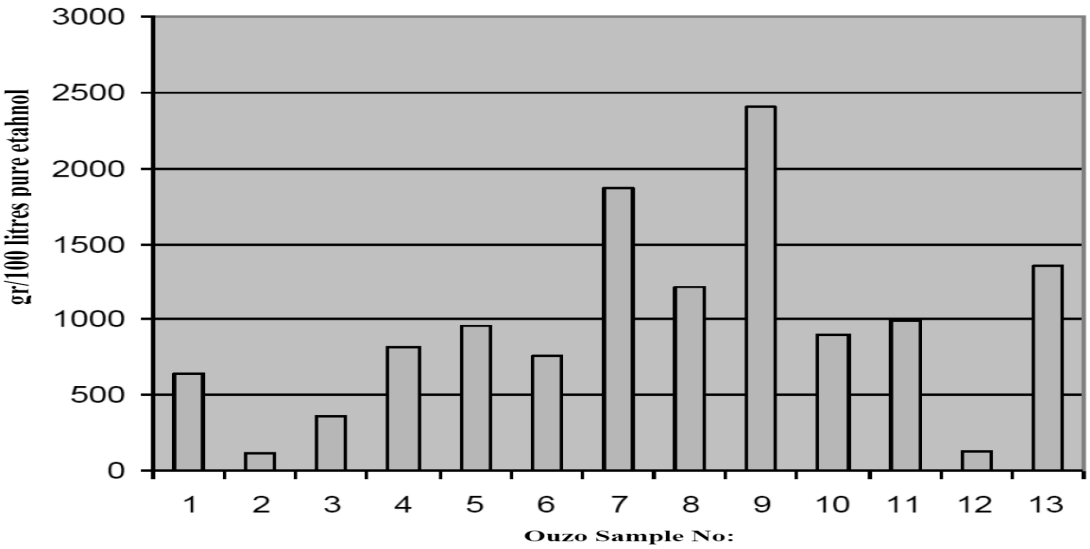

Figure 2. The concentration (grams/100 pure ethanol) of trans-anethole in various ouzo samples

The data presented in Figures 1,2 mark a substantial variation in concentration of trans-anethole between different samples. Particularly, the concentration of trans-anethole varies in the range of 100-2500 grams/100 litres pure ethanol. However the majority of the samples fall in the range 500-1500 grams/100 litres pure ethanol. This fact implies substantially different degree of aromatization by different ouzo producers.

c) Estragole content of ouzo samples

The estragole content of ouzo samples are presented expressed in ppm and in grams/100 litres of pure ethanol in Figure 3,4 respectively.

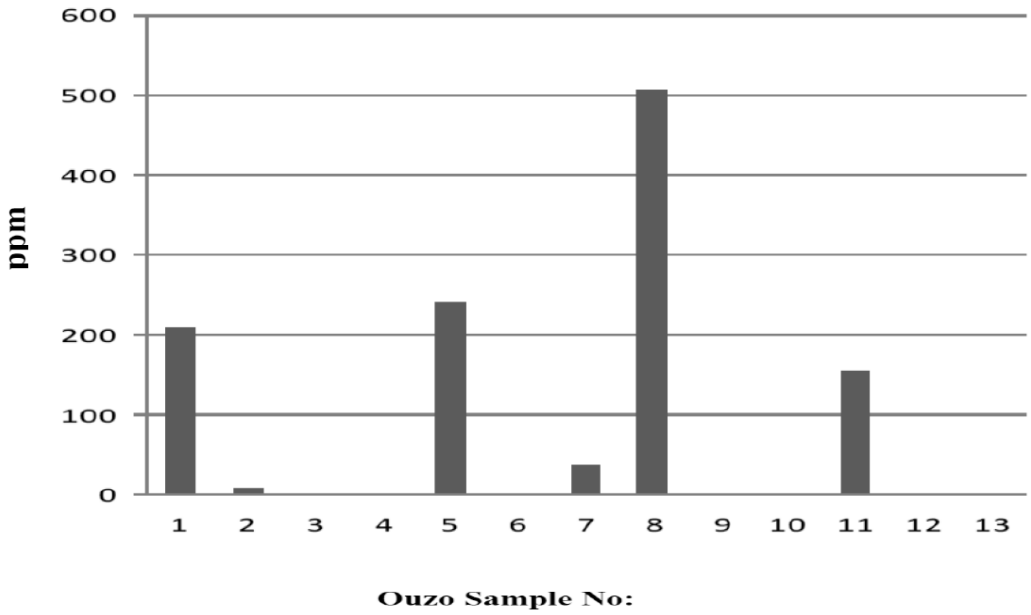

Figure 3. The concentration (ppm) of estragole in various ouzo samples

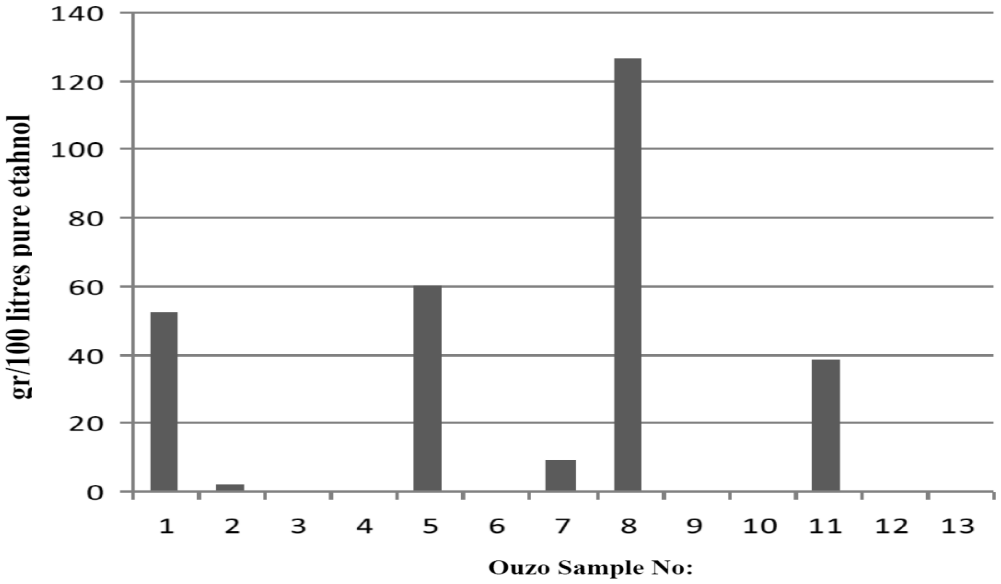

Figure 4. The concentration (grams/100 litres pure ethanol) of estragole in various ouzo samples

From the data presented in Figures 3,4 it is concluded that there is a significant variation in estragole concentration among different ouzo samples with some samples having even zero concentration. The range of the estragole concentration found to be 0-500 ppm or equivalently 0-125 grams/100 litres pure ethanol.

Conclusions

A new improved method GC-MS method was used for analysis of traditional Greek ouzo distillate samples which employed a very long (100m) capillary GC-column achieving better separation. By using

this method the presence of 45 volatile substances was confirmed in the analyzed ouzo samples. According the analysis data methanol was found to be virtually absent in the samples (it was present only in one sample and in negligible concentration) as well as the toxic cis-anethole. However, trans-anethole confirmed as the main ouzo aromatic and found to vary significantly among samples. Similar results were obtained with estragole which also varied in concentration among different ouzo samples and in some of them was absent. The developed analytical GC-MS protocol can be generally used to analyze similar to ouzo distillates like raki etc.

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Frontier research for knowledge-based bio-economies (KBBE)

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The perception that we as citizens of planet Earth should turn to Earth, to life itself, to help economies to develop in a way which should not just enhance the quality of life, but also maintain it for future generations, has pioneered the broad field of thematic research now called "KBBE", where the "knowledge base" includes advances in life sciences and biotechnologies in convergence with other technologies such as nanotechnologies, chemistry and information technologies, and the term "bio-economy" includes all industries and economic sectors that produce, manage and exploit biological resources.

This new holistic approach transcends the narrow confines of scientific disciplines – blending various scientific disciplines, and cutting across policy areas: from research and innovation, to trade and health and consumer affairs. In addition, it involves bringing all stakeholders together to chart a common course into the future which ensures that science will be delivering what people need and complies with an acceptable ethical consensus.

Frontier research in KBBE covers innovative food technologies for producing new eco-efficient competitive bio-based products, innovative animal breeding technologies, as well as non-food uses of crops as sources of new materials such as biodegradable plastics, biodiesel, bioethanol, and biocatalysts. This new type of research is also characterized by responding to global social and economic challenges like food-related disorders, leading to tailor-made foods targeted at specific consumer needs and tastes, sustainability in agriculture/fisheries, climate change, clean biomaterials from renewable bio-resources.

The presentation will highlight examples of selected frontier research in relevant thematic areas of KBBE supported by EU framework projects in recent years.

The study of chamomile effects on the growth of probiotic bacteria, *Bifidobacterium bifidum*, in milk and yoghurt

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The usage of natural antibacterial composition including pharmaceutical plants to keep foodstuffs increases remarkably, in addition to antibacterial properties these compositions are antioxidant, pharmaceutical and flavoring. On other hand, as a targeted food the probiotic products play important role in wholesome food production and too these probiotic compounds with decreasing the risk of heart attacks and improving desirable microbial fluoride in the digestive system have a wonderful effect on the consumers healthy.

This study was done to evaluate the effect of Chamomile on probiotic bacteria activity including lactobacillus acidophilus and Bifdobacterium bifidum as the initiator bacteria of probiotic yogurt and fermentative milk. 0.33 gr. Liophylized bacterium of bifidobacterium bifidum and lactobacillus acidophilus were added separately to one liter of sterilized low fat milk in order to define the effect of different doses of Chamomile (Zero, 0.2, 0.4 and 0.6 percent) on probiotic Bifidobacterium bifidum and lactobacillus acidophilus growth in first (milk) and second (probiotic yogurt) steps. A mixture of both bacteria in amount of 0:165 gr. were tested by virtue of above steps. The samples were examined by virtue of the pH, acidity, microbial counting and culture and coloration in the greenhouse period and durability. The products were evaluated sensorially in tenth day. The questionnaire results were examined and analyzed in descriptive statistical test by SPSS software.

The results indicated that the samples with 0.6 percent concentration of Chamomile had best flavor, durability and color and considering the low fat milk was used to produce yogurt the latter was fatty. Biologic capability of the probiotic bacteria were counted in direct and culture on 'MRS' environment method. The numbers were acceptable in the 20 day period and also the samples with and without Chamomile had tangible difference; the 0.6 percent concentration had increased the yogurt durability and accelerated bacteria fermentation and growth in all cases except in Bifidobacterium bifidum milk and when the mixture of both bacteria were used the bacteria growth speed and numbers of the colonies resulted from their culture increased, too and the colonies resulted from the bacteria where observed round and clear in milk and wide and dentate in yogurt. Also like extract and essence when sugar was added the bacteria growth increased. A sensory evaluation was done at tenth day of production. In statistical descriptive test, the results of questionnaire were obtained by SPSS software system and Crosscal voice test concerning the effect of four fold groups on variables such as scent, color, resistance, and flavor, which there was a meaningful difference between sample's colors ($P<0.05$).

Nanoemulsion-based delivery systems for the encapsulation of essential oils to be used as antimicrobials in foods

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Abstract

Essential oil (EO) components (phenols, terpenes or aldehydes) can be encapsulated into nanometric delivery systems to be incorporated into fruit juices, to increase the antimicrobial activity, reducing the amount of antimicrobial compound required and therefore minimizing the impact on the quality attributes of the final product.

Nanoemulsions based delivery systems, fabricated by high pressure homogenization with food grade ingredients, were able to protect the EO components and, thanks to their subcellular size, to activate the mechanisms of passive transport through the cell membrane thus increasing the observed antimicrobial activity.

The effect of the delivery systems on the antimicrobial activity of different EO components, such as carvacrol, limonene and cinnamaldehyde, was investigated by determining the antimicrobial activity for three different classes of microorganisms (*Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, *Escherichia coli*).

The incorporation of EO components into nanoemulsion-based delivery systems resulted in a significant increase of the antimicrobial activity in comparison to the pure compounds, which significantly depended on the delivery systems physicochemical properties, such as mean droplet size and emulsifier composition.

The application of the most efficient antimicrobial nanocapsules was tested in pear and orange juices inoculated with *L. delbrueckii*. Due to the higher antimicrobial activity of the nanoencapsulated compounds, low antimicrobial concentrations are required for a bactericidal action under accelerated aging at 32°C, with minimal alteration of the organoleptic properties of the juice.

Introduction

The inhibition of pathogens or saprophytes through environmental control represents an approach to food preservation that can compete with aseptic handling and packaging, mechanical removal of microorganisms by washing or filtration and destruction of microorganisms by physical or chemical sanitization and finally (Davidson, Sofos, & Branen, 2005) thanks to the contribution benefited most from the recent developments in nanotechnology.

In particular, inhibition of microbial growth can be achieved through the addition of natural compounds, such as essential oils, with an inhibitory or bactericidal/fungicide activity (Weiss, Gaysinksy, Davidson, & McClements, 2009). Unfortunately, the addition to complex food systems of chemically reactive species, such as antimicrobial agents, can cause negative effects on the physical stability or integrity of the food chemistry as well as the degradation of the biological activity of bioactive compounds (McClements, 1999). Therefore, very often, the amount of antimicrobial compounds needed to inhibit microbial growth within the limits imposed by food regulations, are exceeding the amounts that ensure a minimal alteration of the qualitative properties of the product (Weiss, et al., 2009).

Nanoencapsulation of essential oils represents at the same time an efficient approach to increasing the physical stability of the active substances, protecting them from the interactions with the food ingredients and to increase their bioactivity, because of the subcellular size that induces passive mechanisms of absorption through the cell membrane. In addition, encapsulation can increase the concentration of the bioactive compounds in food areas where microorganisms are preferably located, for example water-rich phases or liquid-solid interfaces (Weiss, et al., 2009).

Proof of these concepts was given by the improvement of the antimicrobial activity of essential oils when encapsulated into liposomal delivery systems (Liolios, Gortzi, Lalas, Tsaknis, & Chinou, 2009; Gortzi, Lalas, Tsaknis, & Chinou, 2007), or by the encapsulation of eugenol and carvacrol into nanometric surfactant micelles also resulted in enhanced antimicrobial activity (Gaysinsky, Davidson, Bruce, & Weiss, 2005).

This work with the issues of formulation and fabrication of nanoemulsion-based delivery systems for the encapsulation of essential oils, with the aim of improving their antimicrobial activity. In particular, different essential oil components will be tested and will be compared with a terpenes mixture extracted from *Melaleuca alternifolia*.

Materials and Methods

Emulsion preparation and characterization

The tested antimicrobial compounds are Carvacrol, D-Limonene and Cinnamaldehyde (Sigma-Aldrich, Germany) and a mixture of terpenes extracted from *Melaleuca alternifolia* (provided by Istituto Superiore della Sanità, Italy). In the emulsion fabrication, sunflower oil (Sagra, Italy) was eventually added to the essential oils, while soy lecithin Solec IP (a generous gift from Solae Italia s.r.l., Italy), Tween 20 and glycerol monooleate (Sigma-Aldrich, Germany), CLEARGUM® CO 01 (a generous gift from Roquette, Italy), pea proteins F85M (a generous gift from Roquette, Italy), sucrose esters P-1670 (a generous gift from Prodotti Gianni, Italy) were used as emulsifying agents.

The sunflower oil or essential oil-in-water nanoemulsions were prepared using a High Pressure Homogenization (HPH) technique. Primary emulsions were obtained by High Shear Homogenization (HSH), using an *Ultra Turrax T25* (IKA Labortechnik, Germany) at 24000 rpm for 5 min. The primary emulsions were then subjected to HPH in a Nano DeBEE Electric Bench-top Laboratory homogenizer (BEE International, USA) five times at 300 MPa.

Droplet size distribution was determined by photon correlation spectroscopy (PCS) at 25°C (HPPS, Malvern Instruments, UK). From the PCS data, the average droplet diameter (z-average) was determined. Prior to any measurements being taken, the samples were diluted with bidistilled water to a suitable concentration. Each measurement was replicated twice, with the means and the standard deviations being calculated.

Microbial inactivation tests

Experiments were carried out on three different microbial strains grown to the stationary phase in an aerated incubator (Haeraeus Instruments): *Saccharomyces cerevisiae*, *Escherichia coli*, *Lactobacillus delbrueckii. S. cerevisiae* yeast was grown in MRS broth (Oxoid, UK) at 32°C for 48 h, *E. coli* in Tryptone Soya broth (Oxoid, UK) at 30°C for 18-24 h, *L. delbrueckii* in MRS broth at 32°C for 48 h. Experiments were carried on three different microbial species grown to the stationary phase in an aerated incubator (Haeraeus Instruments): *Saccharomyces cerevisiae* (ATCC 16664), *Escherichia coli* (ATCC 26), *Lactobacillus delbrueckii* sp. *lactis* (ATCC 4797).

S. cerevisiae yeast was grown in MRS (de Man, Rogosa and Sharpe) broth (Oxoid, UK) at 32°C for 48 h, *E. coli* in Tryptone Soya broth (Oxoid, UK) at 30°C for 18-24 h, *L. delbrueckii* in MRS broth (Oxoid, UK) at 32°C for 48 h.

The inactivation tests of the three microorganisms in the presence on encapsulated antimicrobial agents were carried out at 0.1% antimicrobial concentration, which was previously proved to be a sufficient concentration to explicate a bacteriostatic or a bactericidal action (Donsì et al., 2011) in comparison with a control, where antimicrobial agents were replaced by sunflower oil.

The microorganisms, centrifuged at 6500 rpm for 5 min at 4°C, were resuspended in sterile distilled water to a final concentration of 10^4 cfu/ml in test tubes, where the nanoemulsions were added to the desired final antimicrobial concentrations.

The test tubes were hence incubated at 32°C for *S. cerevisiae* and *L. delbrueckii* and at 30°C for *E. coli*. After 2 h and 24 h, the surviving cells were evaluated by standard plate count method. Briefly, 1 ml of each sample was used to prepare decimal dilutions, which were plated in duplicate with Plate Count agar for *E. coli* and MRS agar for *S. cerevisiae* and *L. delbrueckii*. The plates were incubated at 30°C for 24 h for *E. coli* and at 32°C for 48 h for *S. cerevisiae* and *L. delbrueckii.*

Inactivation tests were conducted in duplicate.

Results

Different formulations and fabrication methods were used to produce stable nanoemulsions encapsulating the antimicrobial compounds. In general, the nanoemulsions contained 2% w/w of the active compounds, when essential oil components were used, mixed in the organic phase (sunflower oil), or 5% w/w active compounds, when the terpenes mixture was used.

Table 1 reports all those nanoemulsions, which resulted physically stable over 4 weeks with neither visible creaming nor significant variation of the mean droplet diameter.

Table 1. Formulation and mean droplet diameter (expressed through z-average diameter) of the nanoemulsion-based delivery systems tested.

Sample	Organic phase	Emulsifier	z-average [nm]
CARV1	2% carvacrol in 8% sunflower oil	3% soy lecithin	214±15
CARV2		3% pea proteins	218±22
CARV3		1% sugar ester	123±15
CARV4		0.5% glycerol monooleate 0.5% Tween 20	226±18
LIM1	2% D-limonene in 8% sunflower oil	3% soy lecithin	239±29
LIM2		3% pea proteins	184±6
LIM3		1% sugar ester	169±12
LIM4		0.5% glycerol monooleate 0.5% Tween 20	228±18
CINN1	2% cinnamaldehyde in 8% sunflower oil	3% soy lecithin	196±14
CINN2		3% pea proteins	216±11
CINN3		1% sugar ester	130±13
CINN4		0.5% glycerol monooleate 0.5% Tween 20	293±15
TERP	5% terpenes mixture	1% soy lecithin	74±3

The antimicrobial activity of the essential oils incorporated in nanoemulsion-based delivery systems was tested on three different microorganism species in comparison with control nanoemulsions, where the essential oil was replaced by sunflower oil. In particular, the results are reported as the incremental inactivation caused by the encapsulated formulation, calculated as the ratio between the survival ratio after 2 h of the microorganisms exposed to the nanoencapsulated antimicrobial agents (0.1% w/w concentration of the active compound) and the survival ratio of microorganisms exposed to the control nanoemulsions for the same time. To improve the readability of the graphs, the reported incremental inactivation is limited to 100.

Figure 1 shows that most of the tested nanoemulsion-based delivery systems resulted in a measurable antimicrobial activity on *E. coli*.

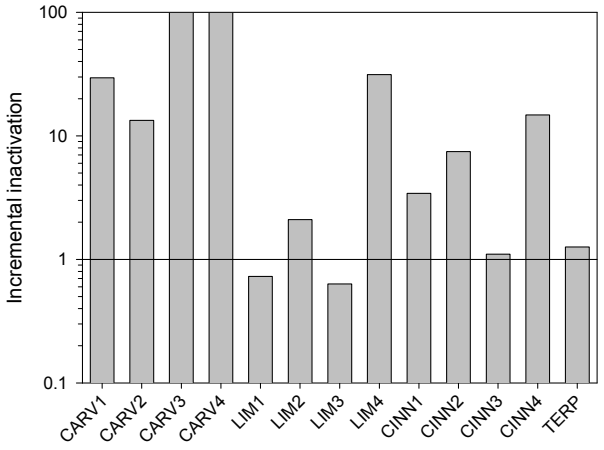

Figure 1. Incremental inactivation of the nanoemulsion-based delivery systems (Table 1) on *Escherichia coli* for a 2 h-exposure at a total antimicrobial concentration of 0.1% w/w.

Remarkably, the increase in antimicrobial activity depended significantly on the antimicrobial compound, but also on the nanoemulsion formulation. In particular, limonene-based formulation did not result to be very active against *E. coli*, with the exception of LIM4 and LIM3 nanoemulsion formulations. On the other side, carvacrol exhibited a very high bactericidal effect for all formulations, followed by cinnamaldehyde and by the terpenes mixture.

The antimicrobial activity of the nanoencapsulated agents against *L. delbrueckii*, reported in Figure 2, showed that carvacrol was again very active (especially CARV3 and CARV4 formulations, with an incremental inactivation >100), while the other delivery systems exhibited a milder but positive antimicrobial activity.

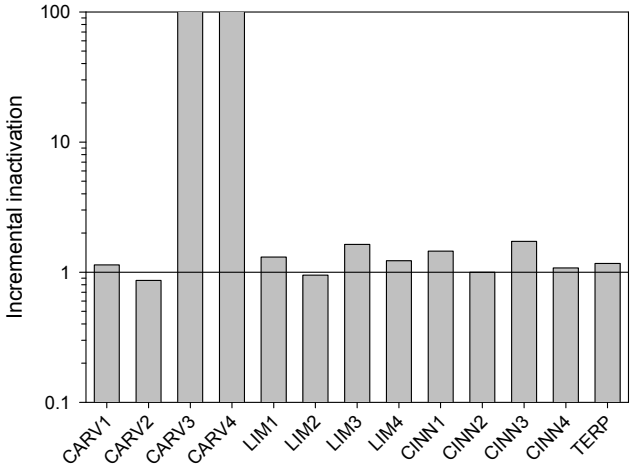

Figure 2. Incremental inactivation of the nanoemulsion-based delivery systems (Table 1) on *Lactobacillus delbrueckii* for a 2 h-exposure at a total antimicrobial concentration of 0.1% w/w.

The results of the inactivation tests on *S. cerevisiae* are reported in Figure 3. In this case, all the tested nanoemulsion-based delivery systems resulted in a measurable and high antimicrobial activity, and only the terpenes mixture exhibited a milder antimicrobial activity.

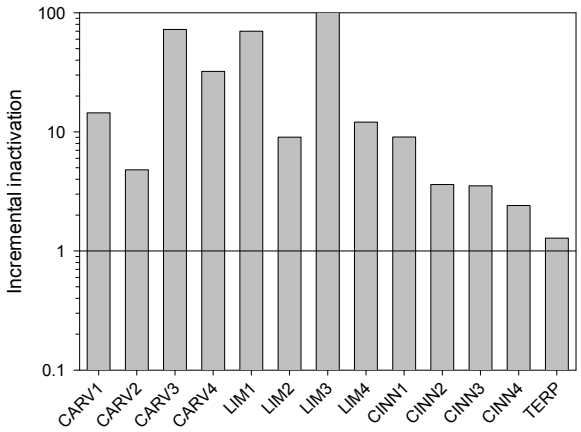

Figure 3. Incremental inactivation of the nanoemulsion-based delivery systems (Table 1) on *Saccharomyces cerevisiae* for a 2 h-exposure at a total antimicrobial concentration of 0.1% w/w.

It must be highlighted that the reported inactivation data are referred to a very short exposure, during which some of the essential oil expressed a bacteriostatic instead of bactericidal activity.

In particular, the antimicrobial activity of cyclic hydrocarbons is limited by their solubility, being available for interaction with cells only those molecules, which are dissolved in the aqueous phase (Sikkema, Debont, & Poolman 1995). Therefore, essential oil components, such as carvacrol, need to be dissolved in concentrations approaching or exceeding their maximum solubility in order to exhibit bactericidal activity (Gill and Holley, 2006). In contrast, limonene, characterized by a solubility significantly lower than carvacrol, exhibits only a bacteriostatic activity unless its concentration in the aqueous phase is increased, for example by favorable partitioning between the the aqueous and a selected lipid phase, or by solubilization within appropriate surfactant micelles.

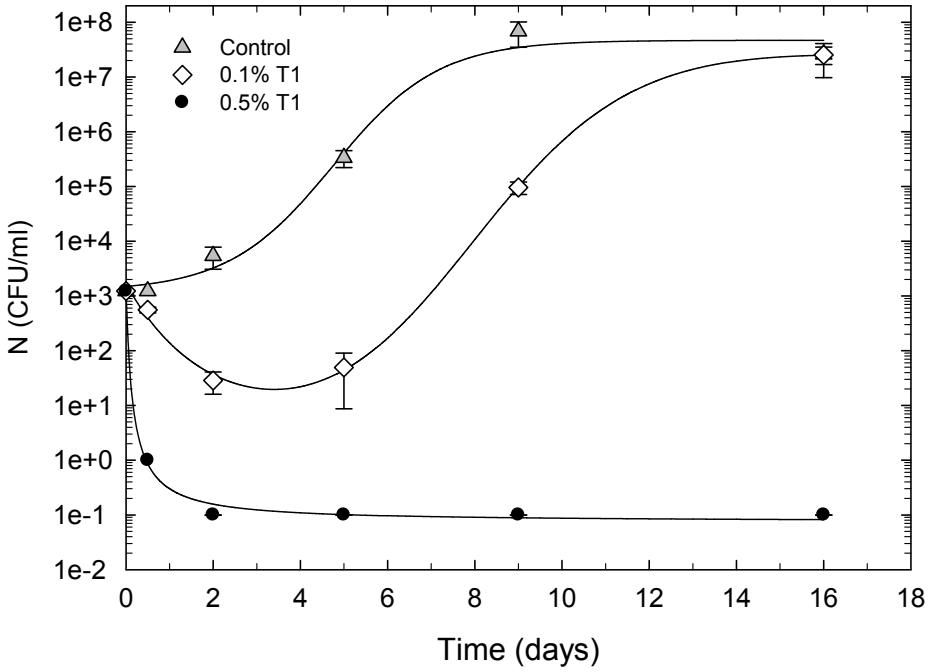

Figure 4. Inactivation curve at 32°C of *L. delbrueckii* suspended in orange juice treated with terpenes nanoemulsion for different terpenes concentrations (0.1% and 0.5% w/w) in comparison with control juice sample.

The TERP formulation was added to orange juice inoculated with *L. delbrueckii* at two different concentrations (0.1% and 0.5% w/w) and the evolution of the microbial population was followed over 16 days. The results of the accelerated shelf life studies are reported in Figure 4, which shows that after 2 days, the total inactivation of the initial microbial load of 10^3 CFU/ml in orange juice was already reached for the terpenes concentrations of 0.5% w/w. At a terpenes concentration of 0.1% w/w, microorganism growth was instead delayed by 5 days in comparison to the control.

Bx and pH were not significantly altered by the addition of the nanoemulsion. This was also observed during the storage period, unless significant microbial growth occurred (data not reported).

Moreover, the impact on fruit juice color of the addition of the terpenes mixture can be considered acceptable at both tested concentrations, with only smaller color deviations being induced. The color remained stable over the storage time for all those systems where no significant microbial growth occurred.

Conclusions

The encapsulation into nanoemulsion-based delivery systems of different essential was investigated as a method to improve the safety and quality of foods through the addition of natural preservatives.

The antimicrobial activity resulted to depend significantly on the essential oil components and on the nanoemulsion formulation. In particular, carvacrol resulted to be the most active essential oil components, while the use of Tween 20/Glycerol monooleate or sucrose esters as emulsifiers resulted to be the most advantageous in nanoemulsion formulations.

The nanoencapsulated terpenes mixture was tested in orange juice inoculated with *L. delbrueckii*. The addition of low concentrations of the nanoencapsulated terpenes was able to delay the microbial growth (0.1% w/w) or completely inactivate the microorganisms (0.5% w/w) while minimally altering the organoleptic properties of the fruit juices.

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Incorporation of olive leaf extract as an antimicrobial agent into polylactic acide film

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Incorporating antimicrobials into a package to prevent microbial growth on foods is limited due to the availability of suitable antimicrobials, new polymer materials and regulatory concerns. Recently, there has been a rising interest on biodegradable biopolymers like polylactic acid (PLA) and natural antimicrobial extracts like olive leaf extract (OLE) for food packaging.

PLA is a hydrophobic, biodegradable and compostable biopolymer, which is produced from renewable resources. Studies on PLA are of current interest not only because of the need to ultimately replace many fossil fuel-derived polymers but also due to the growing global plastic disposal problem. The use of PLA in food packaging has already received wide attention. There have been developments in Europe and in North America that have involved the use of PLA-based packaging for supermarket products. Although PLA-based packages are commercially available, studies on to gained antimicrobial activity to this polymer are rare. There are some studies with PLA using nisin, propolis, lemon extract, thymol, lysozyme as antimicrobial agents for antimicrobial food packaging, but further research is needed to understand its suitability to incorporate and release other natural antimicrobial agents like OLE.

OLE is known with its antimicrobial activity against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus and Staphylococcus aureus*, that all known as food pathogenes. Its antimicrobial efficiency directly related with its polyphenols, namely oleuropein, rutin, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside. To prevent of these food pathogens generations, heat treatments, cold pasteurization techniques, addition of preservatives and some antimicrobials in food formulation or spread these additives on food surfaces may be applied. However, antimicrobial packaging is an innovative way of inhibiting microbial growth on the foods while maintaining quality, freshness, and safety.

Objective of this study was to investigate the effect of OLE on the water vapor permeability (WVP), mechanical properties (tensile strength; TS, elongation; E) of PLA films and antimicrobial activity against *Staphylococcus aureus* (ATCC 25923).

Antimicrobial PLA films were prepared by solvent casting method. OLE was added in the film forming solutions as antimicrobial agent (1-3 g OLE/100 mL solvent) and glycerol (Gly) was used as plasticizer (Gly:PLA 0.5:10, w/w).

Increasing OLE concentration in the film discs revealed a significant increase in the inhibition zone for *S. aureus* ($p<0.05$). PLA film discs containing 0.9-5.4 mg OLE exhibited inhibition zones within a range of 10.75-16.20 mm, respectively. The WVP values of PLA films containing 1-3 g OLE/100 mL film solution did not show a significant difference ($p>0.05$). The E of the PLA films containing OLE changed between 30.77 % and 22.39 %. On the other hand OLE addition did not change TS of the PLA film ($p>0.05$). According to these results, these antimicrobial films may have a potential application for food packaging.

Key words: Olive leaf extract, polylactic acid, antimicrobial packaging

Antibacterial effect of nanosilver particles on pasteurized milk shelf-life

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Abstract

Advanced approaches provided by nanotechnology brought in new opportunities for enhancing human life quality and lead to planning of new and efficient measures to prevent morbidity and mortality rates in the population. Undoubtedly microrganisms (including bacteria) are the major causes of illnesses among people and nanosilver particles provided by the new technology exert tremendous preventive or killing effect on the organism. Milk is a main source of protein, lipid and minerals in human diet. It makes also a good opportunity to the bacteria to growth and then enters the human body. Nanosilver embeded sealed cups can inhibit bacterial growth in milk and may enhance its shelf-life. We prepared %10 and %15 nanosilver composites in the form of cups and filled with pasteurized milk from production line of dairy factory. Control cups with no nanosilver in composition considered as control and treated parallel with the test cups. Microbilogical and chemical tests, routine in milk shelf-line assessments, were done at days 0, 2, 4, 6, 8 and 10 using standard equipments and protocols. Nanosilver composite polymers showed potent inhibitory effects on bacterial growth and prevent chemical changes in stored milk and the milk had increased shelf-life compared to control cups. Nanosilver containers improve shelf-life of dairy products and can apply the technology in marketing section to improve human lives.

Key words: Nanosilver, milk, shelf-life, composite, food microbiology

Introduction

Nanoscience and nanotechnology are concerned with the understanding and rational manipulation of materials at the atomic and molecular level, generally with structures of less than 100 nm in size. Scientifically nanoscience is defined as the study of phenomena and the manipulation of materials at the atomic, molecular and macromolecular scales, where the properties differ from those at a larger scale. Nanotechnology is defined as the design, production and application of structures, devices and systems through control of the size and shape of the material at the nanometer (10^9 of a meter) scale. The electron microscope and, more recently, the development of tools such as probe microscopes, has provided unparalleled opportunities for understanding heterogeneous food structures at the sub-molecular level. This has provided new solutions to previously intractable problems in food science and offers new approaches to the rational selection of raw materials, or the processing of such materials to enhance the quality of food products. This ability to use nanoscience to improve the quality of materials through understanding and refining their nanoscale structures is an example of a form of nanotechnology that has been called incremental nanotechnology.

When the reduction in size of structures leads to step changes in properties, that provide radical new solutions to problems and new commercial opportunities, these types of applications are considered to be examples of what has been termed evolutionary nanotechnology.

The prospect of the use of products of evolutionary nanotechnology in the food area is the area that has engendered most debate. The concern is that if changing the size of materials can lead to radical, albeit useful properties, can we be sure how size will affect other properties and, in particular, the potential toxicity of such materials?. Although the products of nanotechnology intended for food consumption are likely to be classified as novel products and require testing and clearance, there are concerns, particularly

in the area of food contact materials, that there could be inadvertent release and ingestion of nanoparticles of undetermined toxicity. Such concerns need to be addressed because the ultimate success of products based on nanotechnology will depend on consumer acceptance .The recent explosion in the general availability of nanoproducts makes it almost certain that nanotechnology will have both direct and indirect impacts on the food industry.

Importance of milk and milk products in diet

Fluid milk is not only nature's food for a new born infant, but also a source for a whole range of dairy products consumed by mankind. Fluid milk is about 87% water and 13 % solids. The fat portion of the milk contains fat-soluble vitamins. The solids other than fat include proteins, carbohydrate, water-soluble vitamins, and minerals. Milk products contain high quality proteins. The whey proteins constitute about 18% of the protein content of the milk. Casein, a protein found only in milk, contains all of the essential amino acids and accounts for 82 % of the total proteins in milk. Milk also contains calcium, phosphorus, magnesium, and potassium. The calcium found in milk is readily absorbed by the body; Vitamin D plays a role in calcium absorption and utilization. Milk is also a significant source of riboflavin (vitamin B2), which helps promote healthy skin and eyes. Dairy products such as yogurts, cheeses and ice creams contain nutrients such as proteins, vitamins and minerals. Consumption of dairy products has been associated with decreased risk of osteoporosis, hypertension, colon cancer, obesity and insulin resistance syndrome (IRS). The main dietary source of calcium and vitamin D are dairy products. Then milk is healthy food every body need protein, essential electrolytes, fat and other mineral have to use 3 glass of milk in day.

Shelf-life: The ideal milk shows little or no increase in bacteria counts when held under refrigeration; most bacteria that survive pasteurization do not grow at low temperatures. For refrigerated milks that do show an increase, the bacteria that multiply are only those capable of growth under these conditions. While most bacteria prefer warmer temperatures, some bacteria, referred to as *psychrotrophs* ("cold-loving") or *psychro-tolerant*, are capable of growth at 45°F or less. The types of psychrotrophic bacteria most often responsible for rapid milk spoilage and shelf-life failures do not survive pasteurization; thus their presence in milk results from post-pasteurization contamination **(PPC)** due to less than adequate sanitation practices. Although most bacteria that survive pasteurization are not psychrotrophic, a few strains (e.g., thermoduric psychrotrophic spore-formers) will grow slowly and can eventually spoil milk. These organisms can become the limiting factor for shelf-life when PPC is prevented or minimized. The initial day SPC of fresh pasteurized milk is not a good indicator of the numbers of psychrotrophs present. A significant increase in the SPC after 7 days of cold storage is evidence of psychrotrophic growth and suggests that PPC has occurred and that shelf-life will be shortened. Generally, when the SPC exceeds 10 million, milk will become unacceptable due to microbial spoilage. Preventing rapid spoilage and extending the shelf-life of a product requires that post-pasteurization contamination be prevented through a well-designed quality assurance program. It only takes one psychrotroph per container of milk to eventually cause spoilage.

Coliform Bacteria Count: The coliform bacteria ("coli") count is used as an index of sanitation during the handling and processing of milk products. Coliforms are killed by pasteurization, thus when present in milk, they are regarded as post-pasteurization contaminants resulting from poor sanitation. While coliforms are "*indicators*" of PPC, the absence of these organisms does not guarantee that PPC has been prevented. Although the standard is "not to exceed 10/ml," detection of any coliform bacteria, at any time, suggests that there is some point in processing that has been neglected in regard to effective cleaning and sanitation procedures. As a rule, the detection of coliforms in milk will indicate the potential for a shortened shelf-life due to concurrent contamination with psychrotrophic bacteria. Milks with coliform counts exceeding the legal limit of 10/ml are not tasted on subsequent test days in this program.

Aims: to determination of appropriate percentage of nanosilver in polystyrene plate and to increase in pasteurized milk shelf life by nanosilver polystyrene plate.

Antibacterial polymers: One of the vast applications of Nanocomposites is production of multi-purpose antibacterial polymers. Nanocomposite based polymers have well recognised antibacterial, antifungal and antiviral properties, and their presence in the environment are usually green and safe. The best way of their production is well mixing of nanocid composite with polymers like ABS, PET, PP, PE. Suitable master batch of Nanocid composite must be prepared and well mixed with polymers in 10 to 20 percent concentrations. This process includes application or injection of master batch to crude granules of polymers in extruder machines in order to achieve a completely monotonous mixture in desired percentage. About 0.5-0.1 mixed polymers have different industrial, domestic, hospital and many other applications including covering internal body of fridges, making all kinds of water and air filters or plastic dishes. The best method for producing master batch is well mixing of nanosilver composite with polymers and applying it into extruder machines or one can use the extruders are routine to granule producers. Twin extruders are the best choice to rapid and easy production of final products because in these machines mixing of all components is done in one continuous step. If a single extruder with length to diameter ratio of more than 40 cm exists, one can mix the components without needs to master batch preparation.

Methods

Nanosilver particles with desired characteristics were purchased from nano nasb pars company (Tehran, Iran) and applied to food grade polystyrene granules in known percents. The master batch are further processed using machines belong to disposable plate production factory in yasuj. The master bach well mixed with polystyrene granules with %10 and %15 concentrations and put in hoper thermo forming vacuum machine to take 200 ml volumes disposable cups. To determining the cups' antibacterial properties, total bacterial count, coliform and Ecoli detection tests with antibiograms were used.

Study design: Current study was carried out using production line of Dena Kohe dairy product company in Tehran. Totally 300 kg raw milk after primary testing and processing, pasteurized, homogenized, and filled into the cups by rotary fill seal machine. The cups are divided into 3 groups (each group contains 150 cup of %10, %15 or %0.0 nanosilver composite) and treated with proposed plan. All samples kept in market temperature (8-10°c). Microbiological (total bacterial count, coliform and Ecoli detection) and chemical (acidity, pH, alcohol, phosphates) tests were done at days 0, 2, 4, 6, 8 and 10 using randomly selected 25 cups from each group per day.

Measurements: Redigel pretreated dishes containing thin hardener layer and liquid medium containing nutrients with pectin gel sole gelling agent was used to total bacterial counts. Petri film Aerobic Count Plates containing violet red bile nutrients was used to coliform detection. Colorimetery for phosphate and alcohol measurements, titrimetery for acidity and electronic pH metery for pH determination were used**.**

Statistics

One sample t test with known standard values is applied to compare colony counts results. One way ANOVA was used to compare chemical assessments between groups. P value <0.05 considered significant. Mean values from duplicate and triplicate experiments calculated and were entered the statistical inferences.

Results

Table 1. Experimental result of total count

Microbial= total count	Repeatability	Day= 0	Day=2	Day=4	Day=6	Day=8	Day=10
Without antibacterial master batch	1	3000	20000	Non countable	Non countable	In exp 4=non countable	In exp4=15
	2	3200	20000	Non countable	Non countable	In exp 3 = Non countable	In exp4=25
	3	3100	20000	Non countable	In exp 4=5	In exp 4=10	In exp4=30
Contain 10% antibacterial master batch	1	2000	Exp4=0	1200	1100	1 exp 3 10	In exp4=7
	2	2000	Exp4=0	3300	1200	In exp 3 10	In exp4=7
	3	2000	Exp4=0	1300	1200	In exp 3= 10	In exp4=8
Contain 15% antibacterial master batch	1	2200	Exp4=0	20000	270000	Exp 3= Non countable	In exp4=20
	2	2000	Exp4=0	60000	80000	Exp 4=7	In exp4= Non countable
	3	2200	Exp4=0	40000	60000	Exp 4=25	In exp4=50

Table 2. Experimental result of coliform count

Microbial= coli form	Repeatability	Day= 0	Day=2	Day=4	Day=6	Day=8	Day=10
Without antibacterial master batch	1	non grows	non grows	20	100	In exp -1 =non countable	In exp -1 =non countable
	2	non grows	non grows	520	1000	5000	In exp -1 =non countable
	3	non grows	non grows	180	600	3000	In exp -1 =non countable
Contain 10% antibacterial master batch	1	non grows	non grows	non grows	non grows	non grows	non grows
	2	non grows	non grows	non grows	non grows	non grows	non grows
	3	non grows	non grows	non grows	non grows	non grows	non grows
Contain 15% antibacterial master batch	1	non grows	non grows	non grows	non grows	non grows	non grows
	2	non grows	non grows	non grows	non grows	non grows	non grows
	3	non grows	non grows	non grows	non grows	non grows	non grows

Table 3. Results for *E. coli* count

Microbial= *E.coli*	Repeatability	Day= 0	Day=2	Day=4	Day=6	Day=8	Day=10
Without antibacterial master batch	1	non grows	non grows	non grows	Positive Grows	Positive Grows	Grows Indole negative
	2	non grows	non grows	Positive grows	Positive Grows	Grows Indole negative	Positive grows
	3	non grows	non grows	Positive grows	Grows Indole negative	Positive Grows	Positive grows
Contain 10% antibacterial master batch	1	non grows	non grows	non grows	non grows	non grows	non grows
	2	non grows	non grows	non grows	non grows	non grows	non grows
	3	non grows	non grows	non grows	non grows	non grows	non grows
Contain 15% antibacterial master batch	1	non grows	non grows	non grows	non grows	non grows	non grows
	2	non grows	non grows	non grows	non grows	non grows	non grows
	3	non grows	non grows	non grows	non grows	non grows	non grows

Table 4. Results for acidity testing

Chemical=acidity	Repeatability	Day= 0	Day=2	Day=4	Day=6	Day=8	Day=10
Without antibacterial master batch	1	14	14.5	14	15	15.8	21.5
	2	14	14.66	15	14	15.72	21.7
	3	14	14.7	15	14	15.6	21.66
Contain 10% antibacterial master batch	1	13.9	14	14	13	14	15
	2	14	13	14	13	14	15
	3	14	13.76	13.5	13	13.9	14.78
Contain 15% antibacterial master batch	1	14	14	14	14	14.5	45
	2	13.78	12.5	14	14	14.5	45
	3	14	13.87	13.5	14.5	14	45.34

Table 5. Results for pH

Chemical=pH	Repeatability	Day= 0	Day=2	Day=4	Day=6	Day=8	Day=10
Without antibacterial master batch	1	6.74	6.74	6.77	6.86	6.79	6.4
	2	6.74	6.76	6.82	6.81	6.82	6.53
	3	6.73	6.75	6.82	6.80		6.44
Contain 10% antibacterial master batch	1	6.74	6.82	6.85	6.79	6.80	6.82
	2	6.74	6.83	6.82	6.79	6.80	6.80
	3	6.73	6.84	6.82	6..80	6.81	6.82
Contain 15% antibacterial master batch	1	6.70	6.78	6.76	6.73	6.77	5.41
	2	6.73	6.78	6.81	6.71	6.78	5.39
	3	6.72	6.79	6.81	6.71	6.75	5.41

Table 6. Results for alcohol testing

Chemical= alcohol test	Repeatability	Day= 0	Day=2	Day=4	Day=6	Day=8	Day=10
Without antibacterial master batch	1	negative	negative	negative	negative	negative	positive
	2	negative	negative	negative	negative	negative	positive
	3	negative	negative	negative	negative	negative	positive
Contain 10% antibacterial master batch	1	negative	negative	negative	negative	negative	negative
	2	negative	negative	negative	negative	negative	negative
	3	negative	negative	negative	negative	negative	negative
Contain 15% antibacterial master batch	1	negative	negative	negative	negative	negative	positive
	2	negative	negative	negative	negative	negative	positive
	3	negative	negative	negative	negative	negative	positive

Table 7. Results for phosphate

Chemical= phosphates test	Repeatability	Day= 0	Day=2	Day=4	Day=6	Day=8	Day=10
Without antibacterial master batch	1	negative	negative	negative	negative	negative	negative
	2	negative	negative	negative	negative	negative	negative
	3	negative	negative	negative	negative	negative	negative
Contain 10% antibacterial master batch	1	negative	negative	negative	negative	negative	negative
	2	negative	negative	negative	negative	negative	negative
	3	negative	negative	negative	negative	negative	negative
Contain 15% antibacterial master batch	1	negative	positive	negative	positive	negative	negative
	2	negative	negative	negative	positive	negative	negative
	3	negative	negative	negative	positive	negative	negative

Discussion and Conclusion

Current study was designed to evaluate longevity of shelf-life of raw pasteurized milk stored at market conditions in containers made of nanosilver polymer composites of having %10 and %15 nanosilver particles in their compositions.

Zero-day cultured samples had a few number of coliforms or Ecoli in colony counts and up to 2000 CFU in total counts. Phosphate tests for 3 groups were negative. These results reflect good pasteurization process of the milk.

The milk samples cultured in day 2 had no bacterial growth; except for 0.0001 dilution of milk from control cups those results in colony counts under standard limits.

4 day stored control milk samples showed higher numbers in their total colony counts and coliforms were grown more than standard limits, while milk samples from %10 and %15 nanosilver containing composite cups had no coliform or Ecoli in their cultures and their total colony counts were rare.

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Microbiological examination of cow milk under traditional management practices in Ado-Ekiti, Nigeria

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A study on the microbiological quality of cow milk under traditional management practices in Ado-Ekiti was carried out for the purpose of assessing its sanitary level and production condition. The animals (cows) were under the extensive care of the owners (pastoralists). They were entirely fed on range vegetation during grazing and provision of supplementary food was uncommon. Hand milking was usually carried out in the morning before the cows leave their night enclosure.

The milk samples were screened for total bacterial count, total Coliform, yeasts and moulds using the pour plate method. Ten (10) bacterial species, *Bacillus* sp, *Streptococcus* sp, *Staphylococcus aureus, Lactobacillus* sp, *Citrobacter* sp, *Xanthomonas* sp, *Pseudomonas* sp, *Cellulomenas* sp, *Escherichia coli* and *Micrococcus* sp were isolated, characterised and identified in thirteen out of fifteen milk samples analysed in this study. The *Bacillus* sp recorded the highest percentage occurrence (46.7%) isolated from seven (7) samples while *Cellulomenas* sp, *Escherichia coli* and *Micrococcus* sp recorded lowest percentage occurrence (6.7%) each isolated from one sample. Yeasts and moulds were not isolated in any sample.

The results showed that 87% of the raw milk samples were of poor category thus depicting a deplorable state of hygiene and poor production condition during and after milking. The isolation of the various microorganisms in the milk samples is of health significance as some of them may be capable of causing various ailments to consumers which may be fatal. Proper hygiene of the milker, use of clean milking equipment, clean animal environment and subjecting the milk to pasteurization will help to control contamination in milk in order to make them wholesome and acceptable to the consumers at large.

Turkish oils, fats and margarine industry: From margarine to modern margarine

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MÜMSAD (Association of Culinary Products and Margarine Industrialists), established in 2004, represents 15 companies and 32 members at national and international level. Members of MÜMSAD are actively involved in culinary products, margarine, herbal tea and frozen food industries in Turkey. Total market share in Turkish market is approximately 90% for margarine producing MÜMSAD members, Unilever, Ülker, Marsan, Turyağ and Küçükkbay.

MÜMSAD is a member of IFMA, IMACE, EURO FED LIPID and cooperates with AOCS & World Congresses in Turkey, and maintaining fine relationships with M.P.O.B, M.P.O.C, GAPKI, OFI. MÜMSAD is also member of YABİTED (Oils & Fats Science and Technology Association).

The production and import of oily seed, total fat production and margarine production in Turkey are rising where table margarine consumption is decreasing. Per capita total fat & oil & margarin consumption is 21.7 and table margarine generates 2.1 of that.

Margarine industry has improved due to technological and scientific developments. Since margarine is produced from vegetable oils, it does not contain cholesterol. Thus, especially with the technological and scientific developments in the last decade, it turned out to be the modern margarine which does not contain TFA and has a very small amount of SAFA. The American Heart Association (AHA) recommends use of this margarine as a substitute for butter.

The effects of enrichment with olive and hazelnut leaf, and hazelnut green leafy cover extracts on frying oils

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The aim of this research was to evaluate the efficacy of some phenolic extracts to extent use-life and productivity of frying oils. For this purpose the phenolic extracts of olive and hazelnut leaves and hazelnut green leafy cover (çotanak) were prepared and added into canola oil at 200 ppm total phenolics by ***gallic acid equivalence*** (GAE). Control and enriched oils were dough fried for 7 consequtive days at 180 ^{o}C for 5 h per day, and at the end of each day, some oil samples and dough samples were collected and analyzed. Generally, the lowest phenolic content and antioxidant capacity were measured in çotanak extract, although the best performance in frying oils was with çotanak extract as well. There were statistically significant differences among the free acidity, conjugated diens and total polar materials (TPM) measurements. The oil enriched with çotanak extract has even not exceeded the limit TPM value (≤25 %) at the end of seventh day. Also the remaining antioxidant capacity in the frying oil samples were highest in çotanak extract added oils. The viscosity and turbidity values of the enriched oils were a little higher than control sample, but smoke point depletions were lower. There were frying-time dependent changes in the color values of the oils. The fatty acid composition of the seventh day oils and control oil were also measured. Generally *trans* acid formation was lower in enriched oils, especially in hazelnut green leafy cover extract added samples. On the other hand, there was significant decreases in the level of unsaturated fatty acids through frying period, especially in the control oil. Also, there were significant correlations between the measured parameters of free acidity, total polar materials, conjugated dienoic acids, viscosity and turbidity. Smoke point was negatively correlated with almost all parameters. In conclusion, it was shown that plant phenolic extracts may extent stability of frying oils, and among the three extracts studied the çotanak extract was superior and may have commercial value for this type of applications. Definition of the active molecules in the extracts, investigation of the effect of different addition levels and evaluation of similar plant materials in frying conditions are more research topics identified from this study.

Oil structuring with plant sterols mixtures (γ-oryzanol + β-sitosterol)

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Mixtures of plant sterols (i.e. i.e. γ-oryzanol and β-sitosterol) can be potentially used as an alternative to crystalline fats i.e triacylglycerols (TAGs) in oil structuring of food products i.e organogel and emulsion-gels. Crystalline TAGs contain high levels of saturated fatty and that increases the cholesterol level in blood which increases the risk on cardio-vascular diseases. The use of plant sterols in oil structuring has several advantages, for instance they are free of saturated fatty acids and have a lowering cholesterol effects. In pure oil system i.e. sunflower oil, mixtures of γ-oryzanol and β-sitosterol were found to self-assemble in to hollow tubules (~ 7 nm in diameter and 1 nm in wall thickness) forming a transparent and firm organogel. Introducing water the system to form w/o emulsion-gel reduced the firmness of the emulsion dramatically and resulted in a different microstructure. It was found that the water in the emulsion binds to the β-sitosterol molecules forming sitosterol monohydrates which reduced the availability of β-sitosterol for self-assembling with γ-oryzanol and consequently hindered the formation of tubular microstructure. In order to solve this problem, the water availability in the oil phase should be reduced to suppress the formation of sitosterol monohydrate crystals and allow the self-assembly of sitosterol and oryzanol into tubules. This was investigated following two different routes. The first one is based on thermodynamics by reducing the water activity by adding of salt or sucrose to the water phase, whereas the second option is based on kinetics by reducing the transport rate of the water through the oil phase and ultimately to the sitosterol surface by using low polarity oils. The DSC and SAXS results show that reducing the water activity below ~ 0.9, suppresses the formation of the sitosterol monohydrates allowing the formation of self-assembled tubular microstructure. The resulting firmness of the emulsion increased. The polarity of the continuous oil phase, which relates to the solubility of water in the oil phase, strongly affects to the structuring behavior of the system. With low polarity oils, the formation of hydrated sitosterol crystals was hindered and as a result the sterols compounds were able to self-assemble into tubules, which significantly increased the firmness of the emulsions.

Sensitive determination of dioxins and pcbs in food and feed using a tandem quadrupole GC-MS/MS system

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Dioxins and polychlorinated biphenyls (PCBs) are ubiquitous, lipophilic organic contaminants that are globally present at low concentrations in food and feed. Due to their toxicological potencies in humans and animals, rigorous performance criteria for their analytical determination are stipulated by legislation in the European Union and the United States. European legislation currently demands that the confirmation of non compliant dioxin and dioxin-like PCB concentrations in food and feed have to be performed by gas chromatography coupled to high resolution mass spectrometry (GC-HRMS).

In a study, the performance of a tandem quadrupole GC-MS/MS system for a reliable, sensitive and unambiguous determination of dioxins and PCBs in food and feed was tested. Samples of meat, liver and hen eggs as well as feed samples, previously analysed by GC-HRMS, were run on the GC-MS/MS system. The results demonstrate good linearity and response reproducibility over the range of interest as well as low detection levels both for dioxins and PCBs.

Results for dioxins and dioxin-like PCBs (dl-PCBs), calculated as toxicity equivalency concentrations (TEQ), between GC-HRMS and GC-MS/MS are comparable and generally in a range of ± 20%. Moreover, the determination of non dioxin-like PCBs (ndl-PCBs) at concentrations of 1 ng/g product and below can be achieved.

The results of this study indicate that the Agilent GC-MS/MS system has the potential as an alternative confirmatory methodology for the determination of dioxins and PCBs in official food and feed control, pending analytical quality criteria to be set by legislative bodies.

Analysis of the stability of the lipid profile of cocoa butter extracted from preserved cocoa beans using gamma irradiation and phosphine chemical preservative

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Abstract

Dried cocoa beans which weighed 250g, were treated with gamma irradiation at 1.0 Kgy level using ^{60}Co source and another sample of equal weight was treated with phosphine. The treated samples were stored for a period of three months. Butter and cake were obtained from the treated and untreated samples. The physicochemical properties of the products which were Free Fatty Acid (FFA), Acid and peroxide values were obtained. The untreated samples developed increase in the FFA, acid and peroxide values during storage while the treated samples were stable. The irradiated samples compared favourably with phosphine treated samples and it could be concluded that gamma irradiation can substitute for chemical treatment such as fumigation that had long age been used in the post harvest preservation of dried cocoa beans.

Introduction

The major uses of cocoa are in the manufacture of chocolate, chocolate drinks and cocoa based beverages. The butter is used in confectionery, pharmaceutical and cosmetics industries. The powder is used in the dairy and confectionery industries (Minifie, 1970).The qualities of the products (powder and butter) depend upon the series of unit operations that have taken place right from the time of harvesting of the cocoa pod (Ihekoronye and Ngoddy, 1985). After harvest, crops must be transported to the consuming centers which are far away from the production centers. Like any other agricultural products, cocoa beans could be lost to various forms of post harvest spoilage agents. If bag storage is employed, care must be taken in order to make sure that no incipient insect infestation is present on the bags which can later damage the beans.

The most widely used method of controlling infestation is by spraying with chemicals. This method usually leaves residues of chemicals, which may cause health hazard when consumed with the products. Besides, chemicals are becoming increasingly expensive and therefore many developing nations may not be able to afford them. Furthermore, many of these chemicals e.g. DDT are being banned by some of the importing countries (Appiah, 1990, FAO/IAEA 1993). Food irradiation promises then to be the most viable alternative to chemical treatments e.g. fumigation in cocoa beans storage against infestation (Olorunda and Aworh, 1990).

This method had been well documented (FAO/IAEA, 1993) and it is described as a physical method of food preservation (IAEA, 1983). However, the effect on quality parameters is yet to be investigated. It is therefore the aim of this study to evaluate the effect of gamma irradiation on the quality parameters of cocoa beans when preserved by irradiation as a substitute to chemical preservation.

Materials and Methods

Cocoa beans, supplied by Ile-Oluji Cocoa Product Company Limited, Ondo State, Nigeria, were used for the study. 250g cocoa beans were exposed to gamma rays using a ^{60}Co irradiator located at the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, Nigeria. The beans were given an average dose of 1Kgy and an equivalent pack was exposed to phosphine gas generated from phostoxin tablets. Another sample of equivalent weight was kept though untreated.

The treated and untreated samples were kept inside kilner jars and stored for a period of 3 months under ambient conditions. The treated and the untreated samples were processed into products (cocoa butter and powder respectively) by shelling the beans manually to get the nibs which were then milled using a Moulinex blender and the butter extracted using a centrifuge (Centromix) at 2,500 rpm for 40 minutes. Changes in the physicochemical and biochemical properties of the products were assessed throughout the period.

The percentage moisture content of the beans (treated and untreated) were determined by the oven method (AOAC, 1975); percentage fat was determined by Soxhlet extraction method (AOAC ,1975). FFA, Acid and Peroxide values were determined by AOAC (1975) method.

The results were analyzed using ANOVA to determine the level of significant difference while the multiple range tests were adopted to separate the means (Bender et al, 1982).

Results and Discussion

The percentage moisture content of the dried beans increased after irradiation from 5.99% to 6.34% while the phosphine treated samples showed no increase in the moisture content (5.92%). During storage, the moisture content of the control sample increased from 5.99% to 6.16% during the corresponding period. However, statistical analysis of the result showed that there was no significant effect on the moisture content of the beans (Table 1).

Table 1. Effect of irradiation and storage on % moisture content of cocoa beans

	Storage Time (Months)			
Dosage	0	1	2	3
Control	5.99	6.38	6.32	6.16
1.0Kgy	6.34	6.33	6.35	6.61
Phosphine	5.92	5.96	6.01	5.90

This result agreed with that of Takyi (1981) on the preservation of oil palm fruit mesocarp by gamma irradiation which showed an accumulation of moisture in the glass jar on day 1 and concluded that this might be due to uncontrolled respiratory activities of the oil palm fruit. Analyses of the moisture content of the irradiation, phosphine treated and untreated samples showed that irradiation had no significant effect on the moisture content of the beans. It agreed with an earlier report of Appiah et al (1981).

The result of the effect of irradiation on fat is given in Table II and it compared favourably with those treated with phosphine and the control For example, at 0 month it was 52.92%, 52.47% and 52.48% for 1Kgy, phosphine treated and the control respectively, while at the 3rd month of storage, the result was 51.11%, 51.72% and 51.49% respectively.

Analysis of the result showed that irradiation had no significant effect on the percentage fat of the cocoa beans stored for 3 months which is a confirmation of the reports of Appiah et al (1981); Takyi and Amuh (1979).

Table 2. Effect of irradiation and storage on % fat content of cocoa beans

	Storage Time (Months)			
Dosage	0	1	2	3
Control	52.48	50.83	50.66	51.49
1.0Kgy	59.92	50.68	50.25	51.11
Phosphine	52.47	50.75	50.88	51.72

As could be interpreted in Tables III during storage, there was increase in the FFA of the butter of the untreated samples from 0.929% at 0 month to 1.153% 1.165% and 1.135% for the 1st, 2nd and 3rd months respectively whereas there was depression in the values at corresponding period of storage for the treated samples.

For the acid value, the results in Table IV, showed 1.84% at 0 month and increased to 2.294%, 2.318% and 2.258% for the 1st, 2nd and 3rd months respectively while the values were depressed in the treated samples. The results of the irradiated samples compared favourably with those of phosphine treated, and the depression in the results immediately after treatment might be attributed to the control of agent that could cause hydrolysis of the butter, mostly enzymes and microorganism. The result agreed with an earlier report of Appiah (1990).

Table 3. Effect of irradiation and storage on free fatty acid (FFA)of cocoa butter

	Storage Time (Months)			
Dosage	0	1	2	3
Control	0.929	1.153	1.65	1.135
1.0Kgy	0.677	0.733	0.795	0.657
Phosphine	0.621	0.564	0.635	0.650

Table 4. Effect of irradiation and storage on acid value of cocoa butter

	Storage Time (Months)			
Dosage	0	1	2	3
Control	1.840	2.294	2.318	2.258
1.0Kgy	1.092	1.168	1.225	1.218
Phosphine	1.147	1.275	1.188	1.172

As shown in Table V, the untreated sample had an initial peroxide value of 9.3meq/kg which was depressed to 8.93meq/kg and 8.80meq/kg for irradiation and phosphine treated samples immediately after treatment respectively. The values for the treated samples remained stable throughout the period of storage while the untreated samples showed an increase in peroxide value to 9.60meq/kg after the third month of storage. The results obtained still fall within the limit reported in earlier works (Takyi et al 1979).

Table 5. Effect of irradiation on peroxide value of cocoa butter (Meq/Kg)

	Storage Time (Months)			
Dosage	0	1	2	3
Control	9.35	9.43	9.40	9.60
1.0Kgy	8.93	8.93	9.00	8.87
Phosphine	8.80	8.90	8.90	8.86

Discussion and Conclusion

During the storage of cocoa beans, there could be loss due to insects, microorganism, and enzymes. Most of the losses in dried cocoa beans are due to hydrolysis of the lipid causing increased FFA and peroxide values. This was shown when there was an increase in the peroxide value from initial 9.35meq/kg to 9.60meq/kg at the end of the third month of storage while the FFA increased from 0.929% at 0 month to 1.135% at the 3rd month of storage. The acid value also went up from 1.84% at 0 month to 2.258% in the 3rd month.

The peroxide, FFA and acid values are all indices of rancidity of fat. From all indications, the untreated samples had undergone hydrolysis during storage while the gamma irradiated samples and phosphine treated samples were relatively stable in their values. It was also shown that irradiation had no negative impact on the overall quality of dried cocoa beans stored for a period of 3 months.
It can therefore be concluded that irradiation can substitute for chemical treatment in post harvest preservation of dried cocoa beans.

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Research on antioxidative properties of foods

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The important role of diet either in promoting or preventing diseases has long been recognized. Global epidemiological studies correlate the prevalence of certain pathologies to dietary habits and confirm an inverse relationship between the intake of antioxidants and the incidence of many chronic diseases, in particular for the role in protection against cardiovascular, degenerative and proliferative diseases. Last years, antioxidants created a great interest with respect to these positive health effects and promoted the development of many research studies and scientific projects investigating the total antioxidant capacities, total phenolic and flavonoid contents of antioxidant sources, and elucidating the extent of biochemical changes on these antioxidants during industrial and home processing.

In this study, food materials with high levels of antioxidants were investigated in terms of their antioxidant capacity as well as the changes that occurred during processing. Chocolate, which has a high antioxidant potential, as its main ingredient cocoa is particularly rich in polyphenols, was investigated by using samples produced at pilot scale in order to clarify the effect of basic chocolate processing steps including mixing, refining, conching, and tempering on antioxidants.

In another study, the changes in flavonoids from fresh tomato to the end product, tomato paste, which has been produced under industrial conditions, were also investigated. Samples were taken at the beginning and end of each processing step including fruit-breaking, pre-heating, separation of the pulp from skin and seeds, evaporating, pasteurization, and canning. The results indicated that the removal of seed and the skin resulted with a major decrease in flavonoids. It was also observed that breaking step of tomatoes increased the flavonoid content by approximately 120%.

Honey is also rich in phenolic acids and flavonoids, which exhibit a wide range of biological effects and act as natural antioxidants. The consumption of tea beverage with honey was evaluated as a distinctive alternative way and tea infusions containing different kinds of honey (polyflora honey (Semdinli Region-BSE), pine honey (BPI), sunflower honey (BSU), and polyflora honey (a special blend - BSB) were investigated in terms of their total phenolic content, total flavonoid content, and antioxidant capacities. In this presentation, several other important sources of antioxidants that have been analyzed by I.T.U Food Engineering Department under the scope of EU Projects, FLORA and ATHENA, will also be evaluated.

Antioxidative properties of rainbow sardine (*Dussumieria acuta*) protein hydrolysate: optimization using response surface methodology

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Abstract

The protein of rainbow sardine (*Dussumieria acuta*), a pelagic-neritic fish in Persian gulf, was hydrolyzed by Protamex® and the optimal hydrolysis parameters of strongest antioxidant capacity of peptides were obtained using response surface methodology (RSM) by the central rotatable composite design (CRCD). The effects of three independent variables: reaction temperature, time and enzyme to substrate (E: S) ratio on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging capacity of protein were well fitted to a quadric equation. Adjusted R-Squared (91.38), Adequate Precision more than 4 (15.009) and the lambda of box-cox plot evaluate the powerty of the model.The hydrolysate with optimal DPPH free radical scavenging was predicted to be obtained at: temperature of 54.37 ◦C, time of 107.21, and E:S ratio of 69.49 AU/kg protein and showed 49.74% free radical scavenging capacity. Results indicated that Rainbow Sardine protein hydrolysate might be considered as a potential source of nutraceuticals and pharmaceuticals in the future.

Key words: Rainbow sardine, Persian Gulf, Protamex, antioxidant, free radical, pharmaceuticals

Introduction

Free radical mediated lipid oxidation is considered to be one of the main limiting factors for the quality and acceptability of foods during processing and storage. To prevent oxidative deterioration of foods it is important to inhibit the oxidation of lipids and the formation of free radicals occurring in the foodstuff and living body. To tackle the problem, antioxidants, both natural and synthetic ones, have been used widely (Khantaphant et al., 2011). Numerous synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, and t-butylhydroquinone are commonly used to retard lipid peroxidation in food. However, their applications are restricted due to potential risks related to health. Therefore, research aiming to identify new antioxidative compounds is important, and the search for effective natural antioxidants is of great interest to researchers (Kim et al., 2009).

Enzymatic hydrolysis of food proteins is an efficient way to recover potent bioactive peptides and protein hydrolysates have been shown to have potential for nutritional or pharmaceutical applications (Wergedahl et al., 2004). Protein hydrolysates from plant and animal sources have been reported to possess strong antioxidant activities, such as capelin (Shahidi et al., 1995), chickpea protein hydrolysate (Li et al., 2008), squid skin collagen (Nam et al., 2008) and loach (You et al., 2010). However, antioxidative peptides from marine food sources are gaining attention as new antioxidative alternatives in the last few years (Rajapakse et al., 2005; Qian et al., 2008).

Worldwide, total landings of small pelagic fish (sardine, mackerel and horse mackerel) are about 4 million tonnes (Dumay et al., 2006). The Sardine fish catching in Persian Gulf, Iran, lead to about 21419 tons annually (Iran Fisheries Organization, [IFO], unpublished data, 2009), which is mostly used as fish meal. Many fish species are under-utilized due to some technological inconvenience, making them more suitable for animal feed than food supplement. But it seems that enzymatic hydrolysis is a good way to recover bioactive peptides from this marine source. The objective of the present work was to investigate the effects of reaction parameters (temperature, time and enzyme to substrate (E: S) ratio) on free radical (DPPH) scavenging activity and optimize the enzymatic hydrolysis condition for the highest antioxidant capacity of the rainbow sardine (*Dussumieria acuta*) protein hydrolysate (RSPH) using Protamex ® treatment.

Material and Methods

Fish (average weight, 47±6 g) were prepared from Jask port in Iran and was freeze in frigidity tunnel. The fish stored at -20°C until use. The protease enzyme, Protamex ®, prepared from Iranian branch of Novo Industry, Denmark.

To hydrolysate preparation a batch of whole freeze fish was minced in a lab-scale blender (Hootkhash Co.Tehran, Iran) and then divided in to 250 ml Erlenmeyer's flasks. The vessels were heated at 85°C for 20 min to inactivate the endogenous enzymes (Guerard et al., 2001). The heat-treated hash was allowed to cool at room temperature before centrifugation at 10°C for 20 min at 6000 × g. Each run contained 1:1 ratio of 100g of heat-denatured minced fish combined with distilled water was incubated at the specified temperature and time period. The pH of the mixture was adjusted to 8 with 1 N sodium hydroxide. The enzymes were inactivated in 80°C for 15 min (Kechaou et al., 2009).

α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging capacity was measured using a modified method of Shimada et al. (1992). DPPH solution (1.5 ml, 0.1 mM in 95% ethanol) was mixed with 1.5 ml sample at final concentration of protein in 50% ethanol. The mixture was shaken and left for 30 min at room temperature and the Absorbance at 517 nm was measured. For the assay control, ethanol solution was used instead of the sample. BHT at a concentration of 0.02 mg/ml was used for comparison.

Response surface methodology (RSM) was applied to optimize the hydrolysis conditions. Central rotatable composite design (CRCD), with 5 levels of each treatment, and 6 replications at the central point has been used (Table 1). The range of independent variables, enzyme activity (x_1), temperature (x_2), and time (x_3), was adopted from preliminary experiments (data not published). Results for the DPPH free radical scavenging capacity as independent variables are shown in Table 1. A quadratic polynomial regression model was assumed to predict the responses. The model proposed for the response is:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \qquad \text{Equation 1.}$$

Table1. Experimental design used in RSM studies by using three independent variables showing observed DPPH free radical scavenging capacity

Run No.	Actual levels of variable			Radical scavenging (%)
	X_1*	X_2	X_3	
1	30	30	60	7.97
2	90	30	60	18.8
3	30	60	60	34
4	90	60	60	35.2
5	30	30	140	15.3
6	90	30	140	19.4
7	30	60	140	35.2
8	90	60	140	46.2
9	9.55	45	100	27.3
10	110.45	45	100	28.6
11	60	19.77	100	3.1
12	60	70.23	100	43.2
13	60	45	32.73	38.7
14	60	45	167.27	35.8
15	60	45	100	46.6
16	60	45	100	49.4
17	60	45	100	48.5
18	60	45	100	41.5
19	60	45	100	45
20	60	45	100	43

* X_1: Enzyme / Substrate Ratio(AU/kg), X_2: Temperature (°C), X_3: Hydrolysis time (min)

where Y is the dependent variable, β_0 is constant, and β_i, β_{ii} and β_{ij} are coefficients estimated by the model. x_i, x_j are levels of the independent variables. They represent the linear, quadratic and cross product effects of the x_1, x_2 and x_3 factors on the response, respectively. The model evaluated the effect of each independent variable to a response. Analysis of the experimental design and calculation of predicted data were carried out using Design Expert software (7.0.0 trial, Stat-Ease Inc., Minneapolis, MN, USA) to estimate the response values. The optimized design was further validated through different random combinations of parameters to evaluate the usefulness of the design.

Table 2. ANOVA table for response as affected by independent variables during optimization experiments

Factors	SS	df	MS	F	p
Model	3447.67	9	383.07	23.37	< 0.0001
Variables					
(X_1)	62.93	1	62.93	3.84	0.0785
(X_2)	17.04	1	1795.01	109.51	< 0.0001
(X_3)	1795.01	1	17.04	1.04	0.3320
Interaction					
$X_1 \times X_2$	0.93	1	0.93	0.057	0.8164
$X_1 \times X_3$	1.18	1	1.18	0.072	0.7941
$X_2 \times X_3$	2.28	1	2.28	0.14	0.7170
X_1^2	642.47	1	642.47	39.20	< 0.0001
X_2^2	1010.56	1	1010.56	61.65	< 0.0001
X_3^2	165.50	1	165.50	10.10	0.0099
Residual	163.91	10	16.39		
Lack of Fit	116.15	5	23.23		0.1758
Pure Error	47.75	5	9.55		
Cor Total	3611.58	19			

P: Level of significance

Result and Discussion

The effects of different parameters including: temperature, Time and E: S ratio on DPPH free radical scavenging capacity is shown in Table 1. Regression analysis and analysis of variance (ANOVA) of the experimental data were performed for the mathematical model fitting, determination of regression coefficients and statistical significance examination of the model terms (Table 2). Multiple regression coefficients were determined by the least-squares technique in order to predict quadratic polynomial models for the studied response variables. The following regression equations were obtained:

DPPH free radical scavenging activity=-115.12796+0.96395E:SRatio+4.07042 Temperature +0.39231Time -7.58333E-004 E:S Ratio×Temperature + 3.19792E-004 E:S Ratio×Time + 8.89583E-004 Temperature×Time-7.41875E-003 E:S Ratio^2 -0.037217 Temperature^2 -2.11802E-003 Time^2 ANOVA test revealed that the quadratic polynomial models adequately represent responses with the coefficients of determination, $R^2 = 0.9546$. R^2 implies that the regression models explained the reaction well, and the fitted model could explain 95.4% of the total variability within the range of values studied. R^2 value is a measure of how much variation is explained by the model, and in both cases the R^2 of the models were in excess of 90%, suggesting a very good explanation of the variance in the data. The model was considered adequate with satisfactory R^2 values (>4).

The model suitability was tested using the lack-of-fit-test, which was not significant for $p > 0.05$. As the test of lack of fit hypothesis was not significant ($p > 0.05$) in the model equation, the model was fitted to the DPPH free radical scavenging capacity data. A good fit means that the generated models explained the variation in the data adequately. Therefore, these models were adequate for prediction within the range of variables employed. The distribution of residual values in the normal probability plot, which is defined as the difference between the predicted (model) and observed (experimental data) indicates that the experimental points are reasonably aligned with the predicted value. Also the normal probability plot,

Fig.1a shows that the distribution of residual values forms a straight line and residual values are normally distributed on both sides of the line. In further analysis, each of the observed values for the degree of hydrolysis was compared with the predicted value. The parity plot (Fig.1b) shows an acceptable level of agreement.

In order to determine the optimal levels of each variable for maximum DPPH free radical scavenger peptides production, two-dimensional response surface contour plots were constructed. Fig. 2 represented the 2D contour plots for the optimization of maximum DPPH free radical scavenger peptides production. Each figure presented the effect of two variables on the response, while other variable was held at zero level. As shown in Fig. 2, the response increased until Time, temperature and E/S ratio reached an optimum point and then decreased following further Time and E/S ratio increase. That was probably because Time and temperature affected the enzyme hydrolysis activity. In terms of temperature, in this model above 45°C produced high DPPH free radical scavenging capacity. In terms of Time, times between 80 to 120 minute hydrolysis in this model produced high DPPH free radical scavenging capacity. Moderate E/S ratio showed high response and an increase in enzyme concentration was not result in improved DPPH free radical scavenging capacity. Initially the hydrolysis may be releasing the Antioxidant peptides resulting in an increase in DPPH radical scavenging capacity of the hydrolysates. However, further treatment may result in the hydrolysis of DPPH radical scavenger peptides. DPPH radical scavenging capacity may decrease with prolonged hydrolysis, suggesting that initially produced DPPH radical scavenger peptides were subsequently degraded. Our model also indicated that temperature had a major effect on the response.

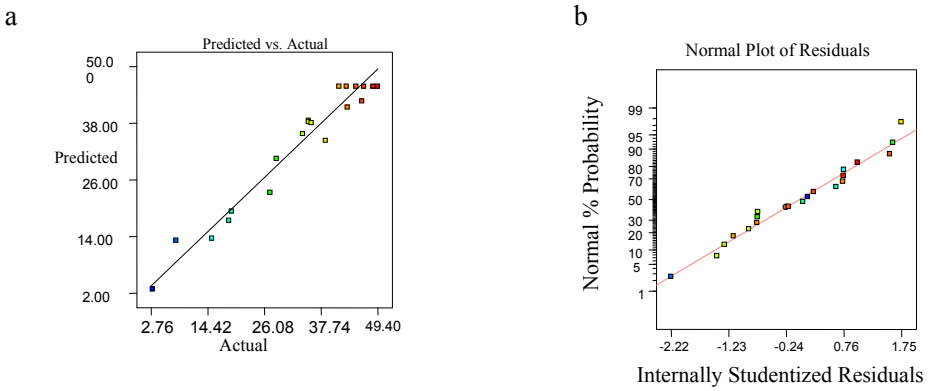

Figure 1. Relationship between the observed and predicted values and parity plot

The optimal conditions were extracted by Design- Expert 7.00 trial version software. The hydrolysate with optimal DPPH free radical scavenging was predicted to be obtained at: temperature of 54.37 ◦C, time of 107.21, and E: S ratio of 69.49 AU/kg protein and showed 49.74% free radical scavenging capacity. To confirm the validity of the suggested mathematical model an additional experiment was conducted under the predicted optimal condition. The DPPH free radical scavenging was 49.12%. The experimental values agreed with the value predicted by the model within a 95% confidence interval. This confirmed that these conditions were optimal for antioxidant peptides production.

Conclusions

Rainbow sardine is one of the important pelagic-neritic fish in Persian Gulf that used for fish meal production. In this study, production of rainbow sardine protein hydrolysate with Protamex was optimized using response surface methodology and antioxidant activity of peptides investigated as response. The results suggested that antioxidant peptide from rainbow sardine protein hydrolysate might be useful as food additives, dietary nutrients and pharmaceutical agents. However, further detailed analysis of their *in vivo* antioxidant activities is needed.

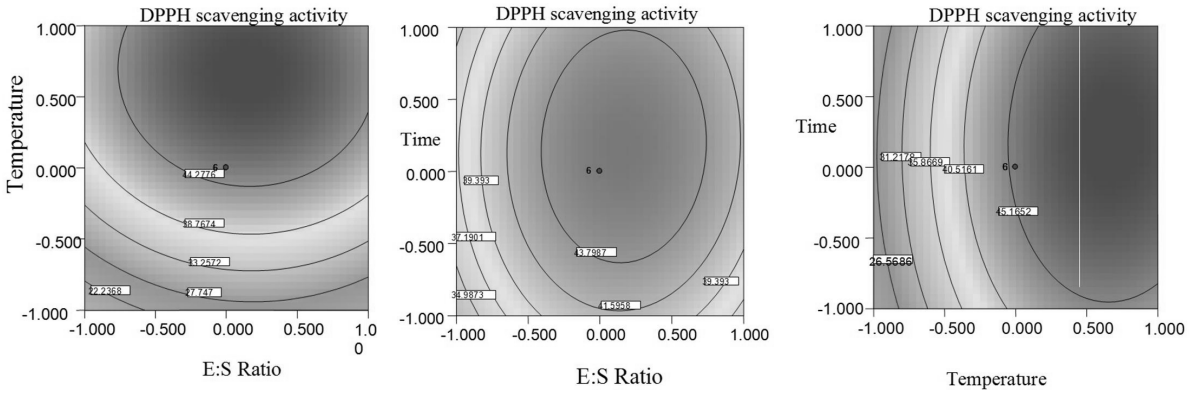

Figure 2. 2D Response surface contour plots for Response as a function of time, temperature and enzyme/substrate ratio during hydrolysis

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Antioxidant activity of probiotic yoghurt fortified with caseinate or whey protein concentrate

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Milk proteins, caseins and whey proteins, have been found to possess antioxidant activity. In the present study, the effect of caseinate (2%), whey protein concentrate (2%) and a blend of 1% caseinate-1% whey protein concentrate on the antioxidant activity of probiotic yoghurt, containing *B.animalis* subs. *lactis*, was investigated. Yoghurt containing 2% skim milk powder was used as control sample. Antioxidant activity of yoghurt samples was determined using DPPH-based method (scavenging of the radical of 2,2-diphenyl-1-picrylhydrazyl), Fe^{+2}-chelating activity and inhibition of hydrogen peroxide (H_2O_2) methods. Control yoghurt sample and yoghurt fortified with whey protein concentrate showed greater DPPH scavenging effect than that fortified with caseinate. Iron-chelating activities were monitored during 60 min. Increase in incubation time significantly improved the antioxidant activities of all yoghurt types. There were no significant differences among yoghurt types in 10 min of incubation time, whereas yoghurts fortified with caseinate and blend of caseinate-whey protein concentrate showed greatest iron-chelating activities by 30 min and 60 min. In the H_2O_2-scavenging activity method two different yoghurt concentrations (0.1 and 0.2 g/mL) were used. Increase in yoghurt concentration enhanced the H_2O_2-scavenging activity in only yoghurt fortified with caseinate. Yoghurts containing whey protein concentrate had higher H_2O_2-scavenging activity among all yoghurt types at 0.1 g/mL concentration. Yoghurts fortified with whey protein concentrate seemed to have higher antioxidant activity depending to the results obtained from DPPH and H_2O_2-scavenging activity methods.

The genetic organization and the stability of plasmid pLP419 responsible for pediocin production at *Pediococcus acidilacitici* s419

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At this study, the genetic nature of bacteriocin production at *Pediococcus acidilactici* s419 isolated from sucuk was investigated. Plasmid curing and PCR search trials showed that 8.8 kb plasmid named as pLP419 was found to be responsible for pediocin production. Plasmid sequencing pointed out that this plasmid involves coding regions; for pediocin operon (3334 bp) consisting papA, B, C and D genes, theta replication origin (repB, 959 bp), plasmid maintenance proteins (orf192, 578 bp and orf114, 344 bp) and tyrosine recombinase (794 bp) respectively. The pediocin operon existing at pLP419 was found 100% homology with the analogous pediocin plasmid pSMB74. However, in this plasmid there were 114 nucleotide differences at 868 bp non-coding region lying between sequences of repB and pediocin operon. After 10 generations, pediocin production stability was decreased 3-fold at s419 strain although there was no difference at appearance of plasmid profiles indicating that the nucleotide differences of non-coding region might have correlation at reducing of antimicrobial activity. These results suggests that the genes responsible for pediocin production at *P. acidilacitici* s419 are coded at plasmid pLP419 having potential as genetic tool for developing starter cultures with antimicrobial activity.

Inhibitory effect of sour pomegranate sauce on some green vegetables inoculated with *E. coli* O157:H7

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Sour pomegranate sauce is one of the most popular natural sanitizers used to give flavor to several foods in Turkey. The objective of this study is to investigate the inhibitory effect of the traditional pomegranate sour sauce and commercial pomegranate sauce on lettuce, spring onion and parsley which were inoculated with acid adapted *Escherichia coli* O157:H7 (ATCC-43895). After detection of the initial flora, lettuce, spring onion and parsley were all inoculated with *Escherichia coli* O157:H7 (4 log-cfu/g) by spot inoculation method. Inoculated samples were kept at 22^0C for 2 hours and then at 4^0C for 22 hours for the attachment of the microorganisms on the surface of the vegetables. After attachment step the inoculated green vegetable samples were treated with pomegranate products for 0, 5 and 10 minutes.

The results showed that both traditional and commercial pomegranate sauce samples caused antimicrobial effects on inoculated microflora of lettuce, spring onion and parsley. Maximum decrease in the counts of inoculated microflora was 4.14 log-cfu/g and minimum decrease in the counts was 0.56 log-cfu/g. It was found that inhibitory effect of all sour pomegranate sauce samples increased by application time.

However, both traditional and commercial pomegranate products were effective on decreasing the numbers of the microorganisms, traditional pomegranate sour sauces were found to be more effective than the commercial pomegranate sauces. These results indicate that pomegranate products used as flavoring and acidifying agent could prevent food-borne outbreaks related to fresh produce.

Key words: Sour pomegranate sauce, antimicrobial, inhibitory effect, green vegetables

Sodium tripolyphosphate and tumbling influence on microbiological quality and sensory properties of döner

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Tumbling, a new mechanical technique, is used for obtaining more tender, juicy, uniform coloured and flavoured meat products as a result of homogenous diffusion of marination sauce including phosphates. However, phosphates improves sensory properties of meat products increasing water holding capacity and preventing oxidative reactions. Additionally, they inhibits microbial growth.

This study was designed to investigate the effects of tumbling and sodium tripolyphosphate (STPP) with marination time on microbiological quality and sensory properties of döner. It was a 2x3x2 factorial experiment, two levels of phosphate (presence or absence of 0.25% STPP), vacuum tumbling (20 minute continuous, 30 minute intermittent [3 minute per 5 minute] or nontumbled) and marination time (day 0 or day 2). Total mesophilic aerobic bacteria (TMAB), coliforms, *Escherichia coli*, S*taphylococcus aureus* counts were determined and presence-absence test for Salmonellae was done to examine the hygienic quality of döner samples. Microbiological analysis was done at day 0 and day 2 for raw samples whereas it was done only at day 2 for cooked samples. At day 2, organoleptic characteristics of döner were evaluated by 8 panelists using 9-point hedonic scale with respect to appearance, color, odour, flavor, texture and general acceptability.

Only tumbling was not effective on sensory properties of döner. It created synergistic effect with STPP. Utilization of STPP in tumbled or nontumbled groups improved colour, flavour and texture properties of döner and the group, continuous tumbled containing STPP, was determined as a favourite group.

In raw döner, both STPP and marination time increased TMAB count ($p<0.01$). Inhibition effect of STPP on coliforms was not observed in continuous tumbled groups. However, this value decreased from 3.65 to 3.37 (log EMS/g) at day 0 and from 3.65 to 3.11(log EMS/g) at day 2 in intermittent tumbled groups by using of STPP ($p<0.01$). At the end of the marination time, coliforms growth was inhibited by STPP with the synergistic effect of tumbling ($p<0.01$). On the other hand, STPP not found effective on *E. coli* ($p<0.01$). *S. aureus* was found <100 kob/g and Salmonellae was not detected from samples. TMAB, coliforms, *E. coli and S. aureus* counts decreased significantly by cooking ($p<0.05$).

As a conclusion, approving of tumbled groups with STPP by panelist was one of the succesfull results of this research. On the other hand, utilization of STPP and tumbling were not found effective on TMAB and *E.coli* counts of döner whereas they were effective positively on coliforms count. Usage of short tumbling processing time and 0.25% of STPP could be a reasons for this result. However, not consumed raw döner and providing of microbial safety by cooking at final product mitigated the effect of this result.

Key words: Döner, tumbling, sodium tripolyphosphate, microbiological quality, sensory properties

Potentialities of acorns for starch production

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Abstract

Quercus suber acorns contain 31.4 % of starch suggesting that these fruits could be a good alternative to conventional starch resources. Starch was isolated from flour by four laboratory scale methods. All the methods involved centrifugation and sieving, but as a first step, in order to increase separation, physicochemical or enzymatic treatments were applied. Different methods produced starch with different properties. Damaged starch content and viscoamylographic profile showed that acorn starch is poorly resistant to any of the tested isolation procedures. The low shear alkaline method using successively three sieves was the most promising one considering the human food usages, since starch showed a high yield, purity and less damaged structure. This method was optimised considering the different centrifugation conditions (velocity and time). A Central Composite Rotatable Design was used as experimental design. Results were treated by Response Surface Methodology. The best encountered centrifugation conditions were 800xg/ 15 minutes. Acorn starch was isolated following these isolation conditions and characterised for by morphological, physicochemical, and gelatinisation properties. Starch showed to be higher in purity and yield, with high contents of amylose and resistant starch. The starch granules were found to be round and oval in shape, with a mean granule size ranging between 9 to 13μm. The gelatinisation temperature was 72 ºC and pastes did not present breakdown, which suggests a high paste stability of acorn starches during heating. Pastes presented a high final consistency and setback.

Introduction

Acorn fruits from *Quercus suber* (QS) are important forestry resources in the Central and Southern regions of Portugal, but they can also be important in Greece, Italy and France (Grove & Rackham, 2001). Most of the fruit production goes to animal feeding, mainly of Iberic pigs. These fruits are also consumed in other European countries (Rakic *et al*., 2006), and there are many different kinds of commercially available processed acorn products, including breads cakes, soups, snacks, noodles and jelly, which are comprised principally of acorn flours (Kim and Yoo, 2009).

Starch as a natural component, contributes to the characteristic properties of food products such as texture, viscosity, gel formation, adhesion, binding, moisture retention, film formation and product homogeneity in different products like sauces, puddings, confectionary, and a variety of low-fat products. Starch is the main component of acorn flour, 31.4% (Correia *et al.*, 2009), suggesting that these fruits can be a good alternative to conventional starch resources, such as cereals and tubers. However, the properties of starches are not only dependent on the starch source but also highly dependent on the history of the starch itself (Wichmann *et al*., 2007) as for instance the extraction procedures. It is known that extraction procedures affect both the chemical composition and physical properties of starch, which justify the interest of studying the most suitable one for each individual raw material.

The present study aimed the optimisation of the isolation procedures and the characterisation of the acorn starch, considering chemical and physical properties and having in mind its potential use on food and non food industry, and so resulting on economic profits.

Materials and Methods

Sample

Quercus suber Lam. (QS) acorns were collected in "montados", located in Idanha-a-Nova (Centre East of Portugal). The acorns were harvested at full maturity. Three sets of 1 kg were randomly collected for each species. Preparation of acorn fruits were performed as describe by Correia (2010).

Chemical analysis

The extraction yield (% of starch produced from flours) was determined. The starch purity was evaluated by determining starch content in the extract by the Ewers polarimetric method (ISO/DIS 10520, 1997). Acorn flour and starch isolated by each method was analysed for: moisture, protein, fat, and ash contents AACC (2000). the total starch content in the flours was determined by the polarimetric method, as proposed by Garcia and Wolf (1972)The colorimetric method proposed by Juliano (1971), was used to determine amylose content, with amylose content being expressed on starch basis. Resistant starch (RS) content was determined following the method proposed by Mun and Shin (2006). Damaged starch content was evaluated by the method proposed by AACC (2000) with some modifications. In this case, reducing sugars were evaluated by the Hizukuri *et al*. (1981) method. All reagents were of analytical grade. All reported values are expressed on a dry weight.

Starch extraction methods

Methods used in starch extraction were selected on the basis of simplicity, efficiency, time consumption and use of low hazard chemicals. The selected methods were based on those proposed by Lim *et al.* (1992) and Perez *et al.* (1993) described by Correia *et al.* (2010). Four isolation methods were tested: Low Shear at Alkaline pH method (LSA); High Shear in Water method (HSW); Low shear enzymatic method (ENZ); Low shear alkaline pH and using successively three sieves (A3S). In all cases, centrifugation was performed in a Universal 16 centrifuge (Hettich Zentrifugen Company, Germany) and isolated starches dried in the aforementioned drying chamber, for two days at 40ºC.

Physical analysis

Scanning electron micrographs (SEM) were taken by an ISI-D 130 scanning electron microscope (International Scientific Instrument). Starch samples were applied on an aluminium stub using double-sided adhesive tape and the starch coated with gold-palladium (80:20). An accelerating voltage of 10 kV was used during microphotography. Pasting of acorn flour, isolated starches and A3S optimised method were monitored in a Brabender (Duisburg, Germany) viscoamylograph at 7%, 8% and 10% starch concentration suspension, respectively, heated from 30 ºC to 95 ºC, hold at 95 ºC for 15 minutes and then cooled till 50 ºC.

Statistical analysis

A Central Composite Rotatable Design (CCRD) was used for the experimental design, performed with k = 2 for A3S - centrifuge velocity/ time as independent variables. No previous reports were found between the responses and independent variables and so the established ranges were: centrifuge velocity (CF) 108xg – 2220xg, centrifugation time (Ct) 10-30 min. The dependent variables studied included starch yield and purity. Data was fit to second-order polynomial equation (1) for each dependent Y variable, through a stepwise multiple regration analysis using Statistic® vs 6 software:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_1x_2 + b_4x_1^2 + b_5x_2^2 \quad (1)$$

where b_n are regression equation coefficients and x_n the independent variables (Kahyaoglu and Kaya, 2006). Based on the predicted model equations surface plots were generated.
The data reported in all the tables and figures are averages of at least three different determinations. A Statistic® vs 6 and Excel®2003 software were used for statistical analysis.

Results and Discussion

Nutritional composition of acorn is shown in Table 1. Acorns presented a high quantity of starch, about 47%, followed by protein, 4.1% (Table 1). Considering the isolation methods, the alkali treatment seems to be quite effective in the reduction of starch fat, protein and ash contents, probably due to an effective protein precipitation and further separation by the use of the sieves. A higher degree of damaged starch was always obtained, being the lower value observed for A3S method. The isolated starches always showed a decrease on amylose content when compared to that found in the original flour, probably caused by the lixiviation/degradation of amylose during the extraction process. Major differences among methods were produced respecting extraction yields. A3S (25.1%) method was the one leading to higher yield. Keeping in mind that acorn starch content in original flours is about 30.4%, and the extraction raw yield was 25.1%, the extraction efficiency was about 82.6%. The extracted starch presented a purity of 95.8%, so 78.6% extraction yield of pure starch was achieved. From what has been stated above, it may be concluded that the methods leading to a higher yield were the A3S. The less efficient was the HSW method probably due to a greater disruption effect on some compounds that are not further separated. This could be observed by SEM images of the isolated starches are shown in Figures 1.

Table 1. Chemical properties of flour and isolated starches

Analysed Parameters	Flour	Isolation Methods				
		LSA	HSW	ENZ	A3S	$A3S_{OPTIMISE}$
Protein (% dwb)	4.1±0.10	2.2±0.01	2.7±0.05	2.8±0.04	0.9±0.01	0.3±0.02
Fat (% dwb)	5.4±0.21	2.1±0.01	2.4±0.01	1.9±0.06	0.8±0.02	0.5±0.01
Fibre (% dwb)	2.8±0.06	ND	ND	ND	ND	ND
Ash (% dwb)	1.7±0.08	0.6±0.04	0.5±0.02	0.3±0.01	0.2±0.01	0
Starch (% dwb)*	30.4±0.81	90.1±2.1	83.6±2.55	91.9±1.05	95.1±0.81	96.1±1.50
Amylose (% dwb)	86.1±1.44	47.6±1.18	48.4±2.33	58.3±2.10	54.4±2.21	57.9±1.33
Damage starch (% dwb)	45.9±0.84	42.9±1.50	48.0±0.71	41.7±1.35	40.1±1.80	23.7±0.74
Resistant starch (% dwb)	43.9±2.01	__	__	__	__	39.2±0.82
Yield (% dwb)	__	18.1±0.55	13.2±0.80	22.1±0.96	25.1±0.55	27.4±0.62

* Starch content in isolated starches is equal to starch purity, expressed in % (dwb). ND– not detested

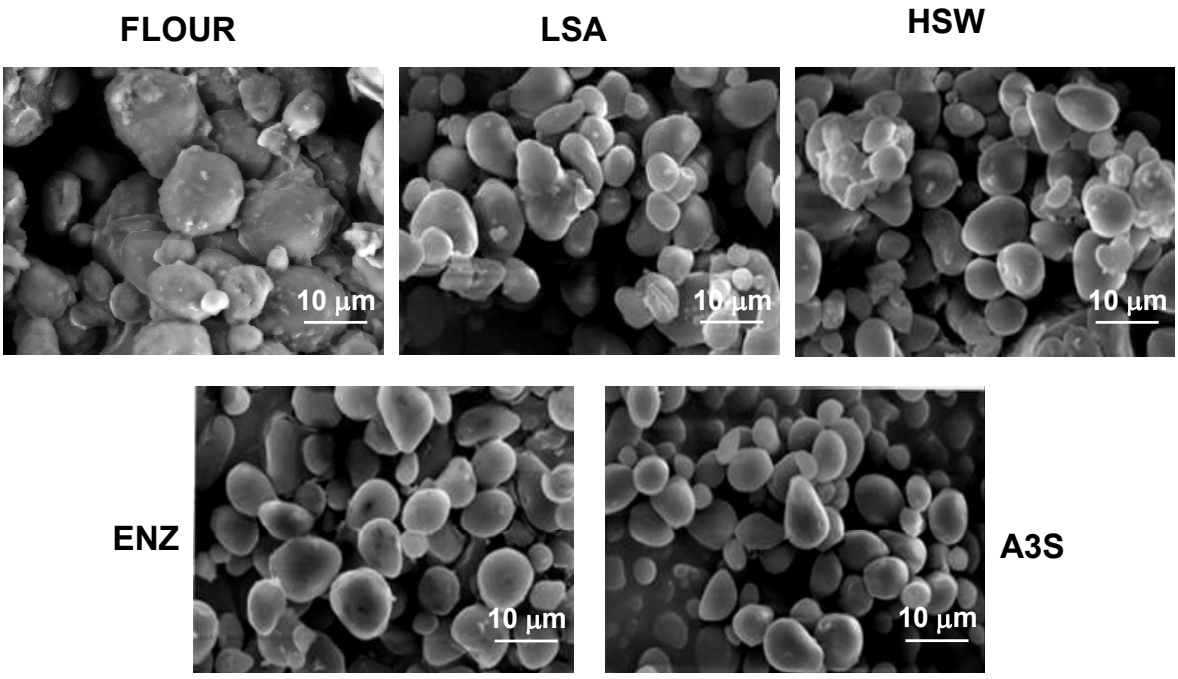


Figure 1. SEM of *Q. suber* flour and isolated starches considering the different isolation methods

The encountered amylographic parameters (Table 2) differences may be explained by changes in the internal structure of the starch granules (Krueger *et al*., 1987), by the degree of damaged starch (Stevens and Elton, 1971), by amylose and amylopectin content (Hung *et al*., 2006) and by the degree of molecular association via hydrogen bonding (Hoover and Sosulski, F., 1985). Generally, acorn starches presented high gelatinisation temperatures (onset temperatures) and the method inducing less damage on acorn starch seems to be the A3S. The gelatinisation temperature is considered as a parameter of crystallite perfection (Huang *et al*. 2007), probably due to high proportion of longer chains in amylopectin (Yuan and Thompson, 1993) which are also responsible for high setback consistency (Srichuwong *et al.,* 2005). No peak consistency was observed for all of the isolated starches, and consequently no breakdown, showing a similar profile to a crosslinked starch suspension (Thomas and Atwell, 1999). This behaviour means that the swollen granules in the starch paste to resist thinning by prolonged heating and mechanical shear (Knight, 1969). This cooked paste stability may show the potential of the starch for use in porridge (Hadimani and Malleshi, 1993). Cold paste consistency increased upon cooling, due to the aggregation of the amylose molecules. This characteristic is estimated by the final consistency (FC). In our study, acorn starches presented high and similar FC. Other authors also reported, as the most distinctive acorn starch pastes properties, the high final consistency and setback (Stevenson *et al.*, 2006).

From the above results it was possible to conclude that the A3S isolation method seemed to preserve in a high degree the starch structure. Thus, it was the chosen one to be optimised, considering the centrifugation conditions, since this unit operation is used to separate protein and fibre from starch and could influence the yield and purity of isolated starch.

Table 2. Pasting properties of acorn flour, and isolated starches

Sample	GT (ºC)	PC (BU)	PT (ºC)	C95ºC (BU)	CAH (BU)	FC (BU)	BD (%)	SB (BU)
Flour	59±1.2	1560±35.0	82.5±1.44	1447±26.0	1215±29.0	2930±49.3	7.3±0.38	1730±43.6
Isolated starches								
LSA	78±1.1	*	*	760±18.3	900±19.6	1760±36.7	nd	nd
HSW	78±0.7	*	*	780±10.6	860±20.5	1320±24.3	nd	nd
ENZ	84±1.5	*	*	820±31.5	880±30.4	1680±40.1	nd	nd
A3S	87±0.6	*	*	780±16.2	880±23.6	1480±20.9	nd	nd
$A3S_{OPTIMISE}$	72±0.6	*	*	1280±34.6	1243±26.0	2540±23.1	nd	1300±28.7

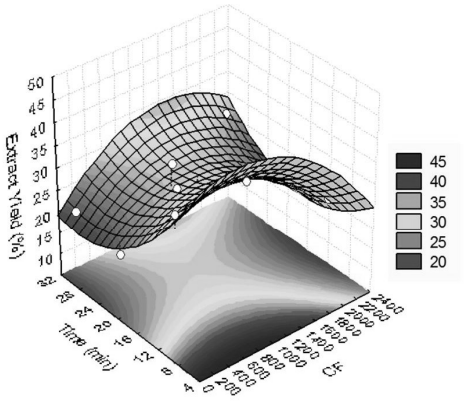
Values are express as mean ± standard error. Consistency is reported as BU (Brabender units). GT, gelatinization temperature; PC, Peak consistency;
PT, peak temperature; FC, C95ºC, consistency at 95ºC; CAH, consistency after holding at 95ºC; FC, final consistency;
BD, breakdown [BD = [(Peak viscosity –Final paste viscosity at 95ºC)/Peak viscosity]x100];
SB, setback [SB = consistency at 50 ºC (BU) – minimum consistency (BU)]
* No maximum peak; nd – not possible to determine

Table 3. ANOVA of yield and purity of acorn starch isolated with A3S method at different experimental conditions

			Factor							
			CF[a] (L)	CF (Q)	Ct[b] (L)	Ct (Q)	CF x C t	Lack of Fit	Pure error	Total SS
A3S	Yield	SS	24.1320	92.8437	243.8640	57.6312	95.3555	39.9937	0.7080	396.1433
		df	1	1	1	1	1	3	1	9
		MS	24.1320	92.8437	243.8640	57.6312	95.3555	13.3312	0.7080	
		F	34.0824	131.1259	344.4164	81.3942	134.6735	18.8281		
		p	0.107999	0.055454	0.034270	0.070277	0.054723	0.167445		
	Purity	SS	1.71855	3.8773	5.76231	8.30579	2.33941	11.26010	1.12500	25.4670
		df	1	1	1	1	1	3	1	9
		MS	1.71855	3.8773	5.76231	8.30579	2.33941	3.75337	1.12500	
		F	1.527599	3.446473	5.122054	7.382922	2.079473	3.336325		
		p	0.433065	0.314549	0.264871	0.224503	0.385999	0.377848		

[a]CF: Centrifugation force; [b]Ct: Centrifugation time

The results of the analysis of variance obtained by the A3S optimised starch isolation method are summarised in Tables 2. Only parameters adjusted ($p \geq 0.05$) by the model are shown. Considering the regression equation coefficients as response parameters the only equations adjusted from RSM were equation showed in Figure 2, with r^2 0.94, related with the yield of starch isolation. In spite of the model presenting a significant fitting ($p \geq 0.05$) for purity, the RSM regression equations did not adjust, suggesting that higher order interaction and/or other variables not considered in the experimental design may have contributed to a better explanation of the data. Both centrifugation force and time had a significant affect on the yield of starch extract. The yield of starch isolation decreased when the centrifugation force was higher than 1500xg (Figure 2). The acorn flour presented a starch content of 31.4%. This means that in ideal extraction conditions the starch extraction yield must be close to this value. The optimum centrifugation conditions for acorns will occur near 800xg during 15 minutes, because for different isolation centrifuge conditions, not only starch was present on the extract but also other compounds. Furthermore, it is very important to guarantee that the centrifuge conditions do not increase the damage of starch granules. Considering the initial starch content in the flours, and the quantity of starch extraction materials, the reached extraction yields and purity were 88.5% and 98.1%, respectively. Comparing the modifications applied to the original method, the yield and purity increased 8.4% and 3.1%, respectively. When we compared the optimised results to those obtained by original method, it was possible to reduce the extraction time (of about 30 minutes) and energy costs. Morphologically, starch granules were found to be round and oval in shape, with a mean granule size ranging between 9 to 13μm.



$Y=56.161719+0.005254CF-0.000007CF2-2.729673Ct+0.049276Ct2+0.000460CF*Ct$; $R^2=0.94$

Figure 2. Extraction yield response surfaces and regression equation coefficients for response parameters for acorn starch isolated by A3S method at different experimental conditions

The gelatinisation temperature of acorn starch, isolated by A3S optimised method was 72 ºC and pastes did not present breakdown, which suggests a high paste stability during heating. Pastes presented a high final consistency and setback. A high setback consistency is associated with cohesiveness of the pastes (Otegbayo *et al*., 2006) and it is generally used as a measure of the gelling ability or retrogradation tendency of starch (Singh *et al*., 2009). Acorn starch presented a high content of resistant starch, and it could be classified as type II (Liu, 2005). It must be emphasised that the high consistency immediately acquired, when the pastes were cooled, suggests possible new applications of this starch as an alternative to the more conventional products.

Conclusions

The best encountered isolation method was the alkaline method at low shear using three sieves (A3S) successively. This method was optimised for centrifugation. Starch produced by those conditions showed to be higher in purity and yield, with high contents of amylose and resistant starch, and low level of

damaged starch. Acorn starch presented high paste consistencies, with higher final and setback consistencies, and did not present a breakdown. Thus, *Q. suber* starch could be classified as to be tolerant to heating and shearing processes. Furthermore, the physicochemical and functional properties of acorn starch, a non-conventional source, suggest that this product may be used as ingredients for foods and other industrial applications.

Acknowledgements

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Multivariate characterization of fresh tomatoes and tomato-based products based on mineral and trace element contents

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The objectives of this study are; (1) to perform a quantitative determination of minerals such as Na, K, P, Ca, Mg, and trace elements such as Se, Fe, Zn, Cu, Mn, Mo and Cd in 80 fresh tomatoes and tomato-based products: tomato paste, tomato juice, minced tomato, tomato puree, grated tomato and dried tomato (2) to evaluate the element composition in relation to product variety and changes in mineral value during processing by inductively coupled plasma mass spectrometry (ICP/MS) (3) to characterize and classify the samples by chemometric analyses - principal component analyses (PCA), hierarchical cluster analyses (HCA) and variance analyses (ANOVA)- and to identify the more healthy product types.

1g samples from each product type were dried in a clean oven at 100 ^{0}C for 24h. 0.5g from these samples were weighed into MW bombs and digested by using a mixture of HNO_3:HCl:H_2O_2=6:2:2. The microwave digestion program applied included the following steps: 250 watt for 15 min ramp 15 min hold, 500 watt for 15 min ramp 15 min hold, and 750 watt for 20 min ramp 20 min hold. The digested sample solution was brought to a volume of 25 ml with deionised water. Triplicate digestions were performed for each sample.

The trueness of the method was verified by analyzing the certified reference material (CRM) of NIST 1573a (tomato leaves). To assess possible contamination during sample preparation, blank samples of ultrapure water were prepared using the same procedure as for the samples. All blank levels obtained were negligible. An internal standard was used for all ICP/MS measurements in order to quantify the elemental composition of the samples and correct for any instrument drift during analysis.

The results of the analyses of minerals, trace-elements and heavy metals are summarised in Table 3. Multivariate analyses were performed using SPSS version 18.0 for Windows (SPSS Inc, Chicago, IL, US).

This study provides information about the similarity of processed foods to fresh foods in terms of mineral and trace element amounts or useful/harmful elements complemented during the production process. This study enables a selection of the most suitable tomato products for human diet and nutrition by determining the product which is most similar in mineral content to fresh tomato, which are tomato juice and minced. Tomato paste and dried tomato contain significantly more Na than fresh tomato due to salt supplementation during the production process to increase shelf-life. A similar effect was observed for Ca in tomato puree and grated tomato, which is supplemented to obtain a more firm structure. Consequently, tomato juice and minced tomato are the best substitute for fresh tomatoes in human diet. The nutritive significance of tomato-based products show difference than fresh tomato but heavy metal levels found practically the same both in fresh tomato and in tomato-based products.

Changes in polygalacturonase activity of date syrup during storage at different temperatures

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Polygalacturonase (PG, EC 3.2.1.15) is the enzyme responsible for the hydrolytic degradation of pectic substances, which results in softening and loss of integrity of the plant tissues. The aim of this study was to evaluate the activity of PG of date syrup stored at the different temperatures for different lengths of time. Dates were weighed, washed and pits removed. Date syrup was prepared by homogenizing the pulp and water mixture for 10 min. The slurry was filtered through cheesecloth with the help of hand press. The filtrate was used for PG determination. A turbidometric assay was used for determination of PG activity. Enzymatic hydrolysis of the substrate (pectin) results in a decrease in absorbance at 340nm, which can be correlated whit PG activity. Results indicated a first order rate kinetics of PG activity changes at 46°C, 25°C, 4°C and -18 °C. The first order rate constants(K) and the half- life ($t_{1/2}$) for these temperatures were 1800 day^{-1} and 0.0.0004 days; 41 day^{-1} and 0.0.016 days; 6 day^{-1} and 0.11 days; and 1.46 day^{-1} and 0.47 days, respectively. From the Arrhenius plot, the activation energy for PG inactivation was 18 kcal/mol. Taken together, the results of this study indicate that PG is more stable at refrigeration and freezing conditions but is dramatically inactivated at temperatures above 25 ^{o}C. It is therefore, recommended that date syrup be stored at lower temperatures to render PG active in order to have a less turbid syrup.

Centrifugal separation technology application for dairy and beverage industries

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The purpose of this study is to explain usage aim of centrifugal seperators especially in dairy industry and to explain centrifugal force in separation aplications. Other than this this study's aim is to explain briefly separator working principles and inside of separators.

The factors effecting separation process will be explained in our study and automatic cleaning facility in modern separator will be described and what brings the automatic cleaning facility in modern seperators will be also explained.

Electro-spraying: a facile technique for lipid coating of foods

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Thin film coating is very popular processing method to produce food with an attractive appearance and improved quality attributes through barrier functionality. Food industry is always in sought of efficient and novel coating methods with key aspects of having high transfer efficiency, evenness and uniform thickness of coating. Electrospraying, attractive but less commonly used in food industry, seems an alternative coating method. It utilizes electrostatic forces to overcome surface tension and generate spray of charged micro droplets. Electrospraying was explored for thin film formation of lipids, e.g. sun flower oil. The focus of this study is on characterization of droplet size and surface coverage by electrospraying in cone jet mode. Food grade sunflower oil was electro-sprayed by single nozzle system on model objects (aluminium and para-film surfaces), which are moving on a conveyor belt. The droplet size analysis and film formation on target surfaces were carried out by image analysis.

As expected, size of electrostatically generated fine charged oil droplets depends mainly on flow rate and conductivity. The experimental data was compared to empirical model for predicting droplet size during electrostatic atomization in cone-jet mode. Measured droplet size was in good agreement with model predictions. Subsequently, droplets of uniform size (30 ± 2 μm) were sprayed on two model surfaces. Droplets were more uniformly distributed on para-film compared to aluminium, while for aluminium; droplet density was relatively high in the middle of spray lane. Film closure was observed after 2-3 passes on aluminium foil, while it appeared not possible to produce a closed film on para-film even after 6 passes. This may be explained by 1) charge accumulation on para-film because of low conductivity and 2) the limited wetting of para-film. Droplet deposition on target surface may be either completely random or influenced by charge of droplets present on surface (a gap filling deposition mechanism). In gap filing disposition, droplets will 'find' a position, which has minimum overlap with neighbouring droplets; resulting in effective surface coverage. These two mechanisms: 1) random deposition and 2) gap filling deposition were simulated with a Monte Carlo approach and compared to experimental observations. It was found that droplet deposition is random, but at higher concentrations, deposition profile is affected by charge interactions between droplets.

These results illustrate that droplet size of electro-sprayed oils can be accurately described from theory. Furthermore, droplet distribution and film closure appears to be affected by accumulated charge at surface of target. These findings contribute to a better understanding of the mechanisms behind thin film coating by electro-spraying. Further focus will be on film characterization with respect to their functionality.

Micronization of carotenoids with supercritical anti-solvent process

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Abstract

Carotenoids, which is fat-soluble plants pigments are compounds, constituted by eight isoprene units joined in a head to tail pattern and most of them have 40 carbon atoms. β-carotene is a primary carotenoid that is necessary in the photosynthetic process, while lycopene is a secondary carotenoid that is not directly involved in the survival of the plant.

Supercritical anti-solvent (SAS) process is one of the microparticle technology with supercritical fluid. In the process, the solid is dissolved in a conventional solvent and the solution is sprayed continuously through a nozzle into the subcritical or supercritical fluid. The dispersion of solution in the fluid leads to an expansion of the droplets and at the same time an extraction of the liquid into the fluid occurs. The solvent power of the conventional solvent decreases dramatically and supersaturation leads to the precipitation of particles. This process is suitable for the fine particles of the carotenoids and absorption to the human body of those compounds is promoted by this micronization.

In this work, the application of SAS process to the micronization of β-carotene and lycopene in supercritical carbon dioxide was studied. Effect of initial carotenoids concentration, pressure and temperature of the process were examined. SAS process was carried out in a semi-continuous cell at pressures of 8 to 12 MPa and temperatures of 40 to 60^{o}C. Initial concentration of carotenoids in the solution were 1 to 8 mg/ml of dichloromethane. Morphology of particle generated was observed by scanning electron microscope (SEM). Different micronized particle shape of β-carotene and lycopene were obtaind. The produced crystal form of micronized β-carotene was plate-like and needle-like shape, while micronized lycopene was calyx-like and leaf-like shape. The pressure and initial carotenoids concentration affected the particle production. High pressure and low initial carotenoids concentration produced small particle size.

Key words: Carotenoids, lycopene, β-carotene, micronization, supercritical anti-solvent

Introduction

Carotenoids are the most common fat-soluble plant pigments in nature which has highly vivid color. There are more than 600 different types, but only around 20 of them are present in the human body, the most important being β-carotene, lycopene, lutein and zeaxanthin [Miguela et al., 2006]. Carotenoids has conjugated double bonds in a molecule and they show colors from pale yellow to vivid red. Those compounds's function is the coloring of vegetables and fruits such as tomatoes and carrots. Because of their chemical structure, they can also behave as natural antioxidants [Mattea et al., 2008]. In the part where the oxygen is generated in the plant body, there is lot of carotenoids. Carotenoids are essential component in the photosynthesis process. These substances are not produced by the human body, and therefore they must be obtained from food. The most important sources of carotenoids in the human diet are green and yellow vegetables, tomatoes, citrics and eggs [Miguela et al., 2008]. Industrial carotenoids are usually crystalline powders soluble in oils and organic solvents, but poorly soluble in water. Due to their antioxidants properties, the carotenoids degrade by presence of heat, oxygen or light easily [Martina et al., 2007]. Lycopene is a natural carotenoid which is present in several fruits and vegetables. The main

source of lycopene in the human diet are tomatoes, in which the concentration of lycopene can be as high as 50 mg/kg, but this carotenoid is also found in watermelons, grapefruits or apricots. Lycopene is not a precursor of vitamin A because it does not have a β-ionine group, but it has antioxidant properties. The capacity of lycopene of fixing singlet oxygen is about three times higher than that of β-carotene. It also has color intensity higher than that of β-carotene [Miguela et al., 2006]. Carotenes (β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin) have vitamin A activity, and these and other carotenoids can also act as antioxidants. Especially antioxidation activity of β-carotene is the highest.

Supercritical anti-solvent (SAS) process is one of the microparticle technologies with supercritical fluid. This process is suitable for the fine particles of the thermal-sensitive, easily oxidized substances as carotenoids, because the low critical temperature of CO_2 allows carrying out the process at near-ambient temperatures and in an inert environment, thus avoiding the degradation of the carotenoid. Owing to the high solubility of organic solvents in supercritical CO_2 (SC-CO_2), solvent-free products are obtained. Absorption to the human body of those compounds is promoted by this micronization. The mixing between the supercritical anti-solvent and the liquid is faster than in conventional liquid anti-solvent processes, thus leading to higher supersaturations and smaller particle diameters. Moreover, the particle size and the particle size distribution (PSD) can be controlled by changes in process parameters [Martina et al., 2007]. For these reasons, supercritical anti solvent processes have been studied for applications including explosives, polymers, pigments, pharmaceuticals and natural compounds [Miguela et al., 2006].

In this work, the application of SAS process to the micronization of β-carotene and lycopene in supercritical carbon dioxide was studied. Effect of initial carotenoids concentration, pressure and temperature of the process on the size and shape of particle generated were examined. SAS process was carried out in a semi-continuous cell at pressures of 8 to 15 MPa and temperatures of 40 to 60^{o}C. Initial concentration of carotenoids in the solution were 1 to 8 mg/ml of dichloromethane. Morphology of particle generated was observed by scanning electron microscope (SEM).

Materials and Methods

Materials and chemicals

Crystalline β-carotene and lycopene with minimum purity of 80% and 90%, respectively, were purchased from Wako, Japan. These carotenoids were used in the precipitation experiment. SEM image of unprocessed β-carotene and lycopene are presented in Figure 1 and Figure 2. Original β-carotene particles are prismatic like crystal with dispersed sizes ranging between 2.2 μm and 32.6 μm. Original lycopene particles are prismatic crystal with size ranging from 19.5 μm to 550.3 μm. Acetone (99%), dimethyl sulfoxide (DMSO) (99%), dichloromethane (DCM) (99.5%) were provided by Wako, Japan. CO_2 (99.5%) was supplied by Uchimura co., Japan.

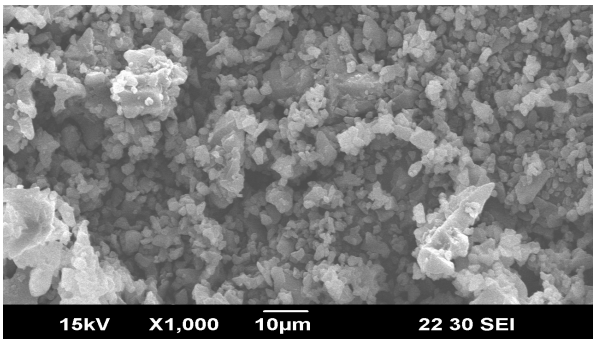

Figure 1. SEM image of original β-carotene particles (1000x magnification)

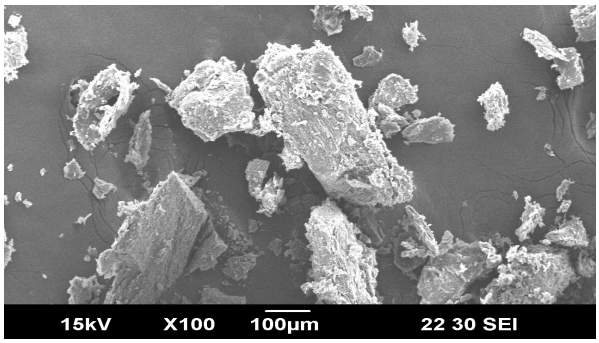

Figure 2. SEM image of original lycopene particles (100x magnification)

Equipment and procedures

SAS process was conducted in a semi-continuous micronization vessel. Figure 3 shows schematic diagram of SAS apparatus. The apparatus included a pump for CO_2 (LC-8A preparative liquid chromatograph, Shimadzu, Japan), a pump for solution (PU-980 intelligent HPLC pump, Jasco, Japan), heating chamber (AKICO, Japan), precipitation vessel (0.5 inch - SUS316 tube, inner diameter: 9 mm, length: 38.5 cm, volume: 24.5 cm^3), nozzle (1/16 inch tube, inner diameter: 0.8 mm), filter (0.5 μm, Swagelok) and back pressure regulator (AKICO, Japan).

A typical experiment was carried out as follows: supercritical CO_2 was introduced in the micronization vessel until the desired pressure and temperature conditions are reached and maintained constant. Afterwards the carotenoids solution was injected with the desired flow rate until an amount of solution has been processed. Then, supercritical CO_2 flow was remained constant to eliminate the remaining organic solvent from the particles. Finally, particles from micronization vessel and filter were collected after the depressurization. The experiment was carried out at pressures of 8 to 12 MPa and temperatures of 40 to 60°C. Initial concentration of carotenoids in the solution were 1 to 8 mg/ml of dichloromethane. Supercritical CO_2 and solution flow rate were 20 and 0.5 ml/min, respectively. Table 1 shows the detailed experimental condition.

Micronization yield

The micronization yield was evaluated considering the amount of micronized powder collected in the micronization vessel. The percentage of micronization yield was calculated by the ratio between the mass of carotenoids collected in the micronization vessel and filter after each assay and the mass of carotenoids present in the DCM solution added to the micronization vessel at each experiment.

Analysis and characterization

Micronized particles were analyzed by a scanning electron microscope (SEM) model JEOL JSM-6390LV to determine particle morphology and shape. Particle size and size distribution were measured by Image J software, using at least 100 particles collected at each experiment.

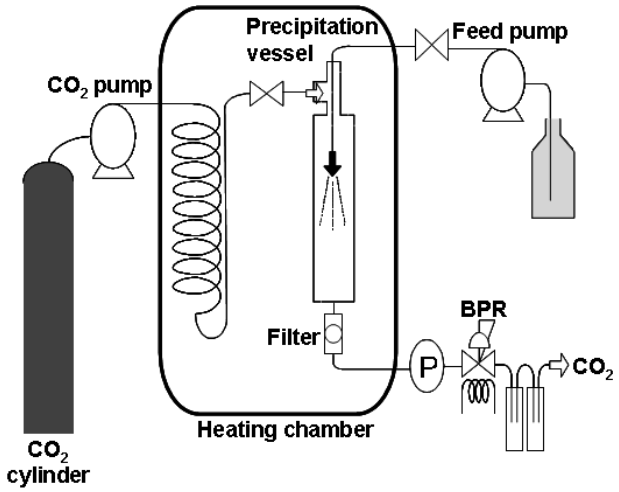

Figure 3. Schematic diagram of the SAS process

Table 1. Experimental conditions

Solvent dependence test (β-carotene)	Acetone, DMSO, DCM
Concentration dependence test (40°C, 8 MPa)	1, 8 mg/ml
Pressure dependence test (40°C, 8 mg/ml)	8, 12 MPa
Temperature dependence test (8 MPa, 8 mg/ml)	40, 60°C

Results and Discussion

Selection of solvent

A suitable solvent was chosen from acetone, DMSO, and DCM in the SAS process of β-carotene at pressure of 8 MPa and temperature of 40°C. As a result, the particles were obtained in the precipitation with DCM, though β-carotene crystal was not observed in the experiment with acetone and DMSO. Because acetone was a good solvent to the carotenoid, it was not possible to deposit the β-carotene. Thus, the carotenoids, SC-CO_2 and solvent were discharged as a homogeneous phase flow. The discharged β-carotene was confirmed from a vent as the evidence. On the other hand, β-carotene in DMSO solution was presented in liquid form after the process. Apparently DMSO was not volatilized easily and was remained in vessel because the solubility of DMSO in SC-CO_2 is very low, moreover boiling point of DMSO is high. However, in the case of DCM, because its volatility was high, and the balance of solubility to both SC-CO_2 and the carotenoid was suitable, the solvent was be fitting to obtain the β-carotene particle in the SAS process. Therefore, SAS process was carried out by using DCM as solvent.

β-carotene micronization

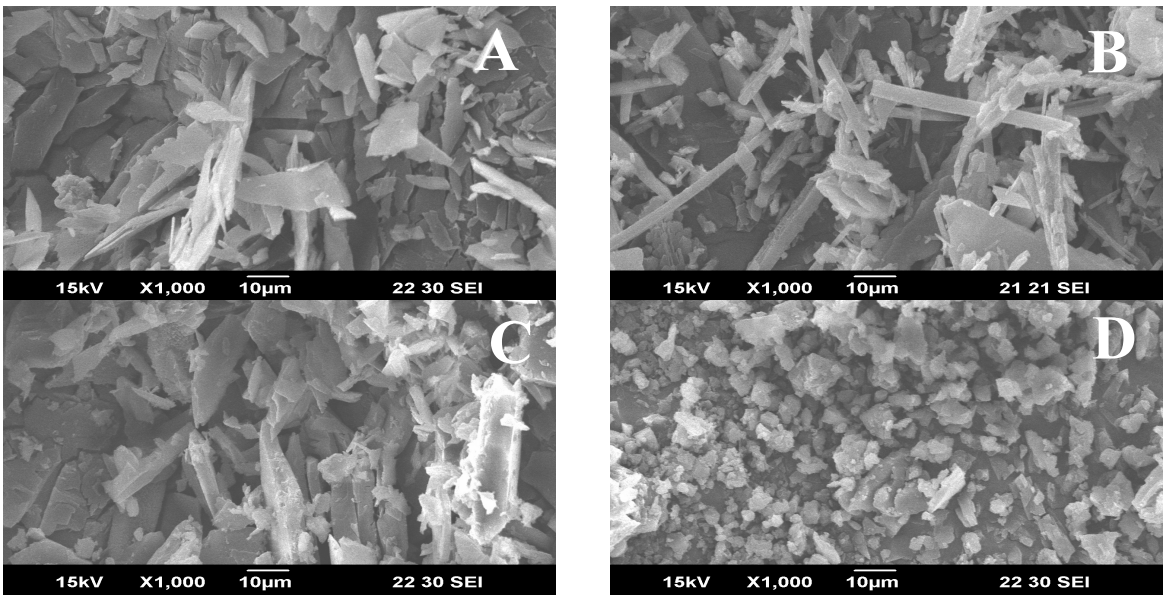

Figure 4. SEM image (1000x magnification) of the processed β-carotene particles precipitated from DCM at various conditions. (A) 1 mg/ml, 8 MPa, 40°C; (B) 8 mg/ml, 8 MPa , 40°C; (C) 8 mg/ml, 12 MPa, 40°C; (D) 8 mg/ml, 8 MPa, 60°C

SAS process of β-carotene in DCM was studied at various initial solution concentrations, pressures and temperatures. Plate to needle-like β-carotene particles were generated by SAS process at various initial solution concentrations [Figure 4(A)-(B)]. Plate-like particles [Figure 4(A)] with size between 7.2 μm and 66.9 μm were precipitated at low initial solution concentration. Higher initial solution concentration resulted needle-like particles [Figure 4(B)] with length ranging from 6.2 μm to 53.5 μm. However, the particle size grew longer than unprocessed particles. At this condition, the increasing initial solution concentration caused increasing precipitation yield from 40% to 75% for 1 mg/ml to 8 mg/ml of solution concentration, respectively.

To evaluate the effect of pressure on the β-carotene micronization, experiments were carried out by changing the micronization pressure from 8 MPa to 12 MPa. This pressure range associated with temperature values (20–40°C) allowed to conducting all the micronization experiments in a homogeneous sub- or supercritical phase according to the phase behavior of the ternary system CO_2 + DCM + β-carotene (Franceschi et al., 2008). Increasing pressure generally caused a raise in the mean particle size and also in the particle size distribution. The strongest effect of pressure was verified at the temperature of

40°C. When the solution was sprayed in the CO_2 bulk phase at the supercritical state, having essentially near-zero interfacial tension, atomization was formed instead of droplets, increasing the two way mass transfer between bulk anti-solvent and solution sprayed, reaching high levels of supersaturation with high degree of nucleation and generating smaller particles with narrow size distribution. The morphology of micronized β-carotene also varied with pressure, changing from needle-like to plate-like form with an increase in pressure. The effect of micronization pressure on morphology and particle size of micronized β-carotene is illustrated in Figure 4(B) and (C). Moreover, micronization yield decreased with increasing pressure from 75% to 33.3% for 8 MPa and 12 MPa, respectively.

Figures 4(B) and (D) show the effect of micronization temperature from 40 to 60°C, respectively, on the morphology and particle size of micronized β-carotene. The morphology of micronized β-carotene was varied with temperature, changing from needle-like to cubic-like form with narrow size distribution. In addition, mean particle size decreased with increasing temperature. At 60°C, size of micronized β-carotene was 1.5 to 16.6 μm. However, at various temperatures micronization yield decreased as increasing temperature from 75% to 58.8% for 40°C and 60°C, respectively. It might be due to the increasing solubility of β-carotene in SC-CO_2, and as the result a part of β-carotene was discharged together with CO_2.

Lycopene micronization

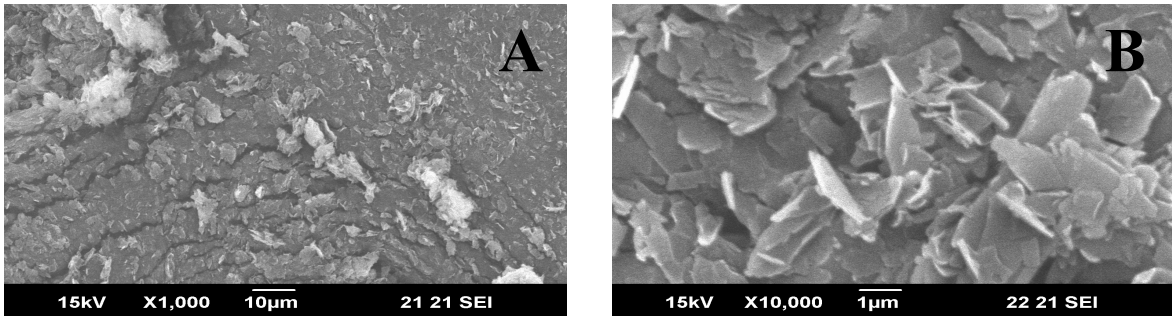

Figure 5. SEM image of the processed lycopene particles precipitated from DCM at solution concentration of 1 mg/ml, pressure of 8 MPa, temperature of 40°C. (A)1000x magnification; (B)10000x magnification

The unprocessed lycopene had considerably lange size as agglomerated particle. However, it became a very fine film crystal after treatment by SAS process. In this work initial solution concentration of lycopene was changed from 1 to 8 mg/ml of DCM. At 1 mg/ml of solution concentration, leaf-like form of particle was precipitated (Figure 5). By increasing initial solution concentration into 8 mg/ml, particle form was changed from leaf-like to calyx-like crystal (Figure 6). Moreover, the particle thickness became thinner as increasing initial solution concentration. However, particle size of micronized lycopene was not influenced by initial solution concentration change. At this condition, micronization yield decreased from 84.6% to 75% with increasing initial solution concentration.

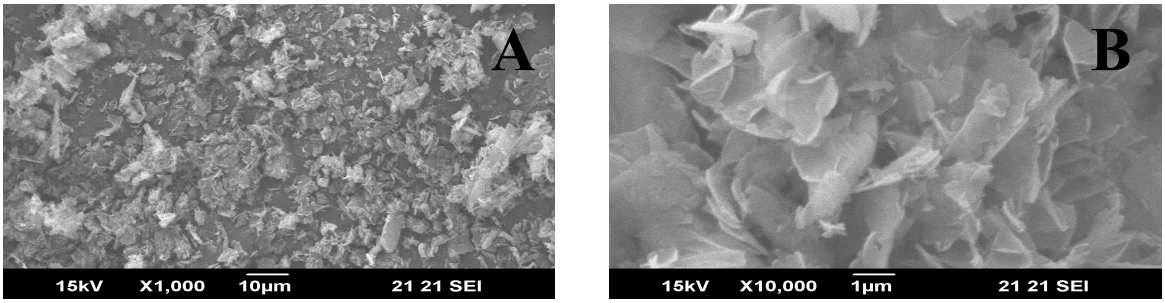

Figure 6. SEM image of the processed lycopene particles precipitated from DCM at solution concentration of 8 mg/ml, pressure of 8 MPa, temperature of 40°C. (A)1000x magnification; (B)10000x magnification

Conclusion

Fine particles were obtained in the micronization with DCM. Plate to needle-like β-carotene particles were generated by SAS process at various initial solution concentrations and pressures. The morphology of micronized β-carotene was varied with temperature, changing from needle-like to cubic-like form with narrow size distribution. In addition, mean particle size decreased with increasing temperature. After treatment using SAS process, very fine film crystal of micronized lycopene was generated.

Acknowledgements

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Optimization of ultrasound assisted extraction of tomatoes processing wastes

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Lycopene, a kind of carotenoid, is responsible for the characteristic red color of fruits and vegetables. Biological and physicochemical properties of lycopene, especially related to its effects as a natural antioxidant make it important component in human diet. Tomatoes and related tomato products are the major source of lycopene. Commercial processing of tomato produces a large amount of waste at various stages. Tomato skin is rich in lycopene so that tomato wastes are important source of it. Traditional extraction methods are time consuming, need high amounts of solvent and may cause deterioration because of heat applied. So that modern, efficient processing techniques like ultrasound assisted and supercritical CO_2 extractions are becoming popular.

In this study, lycopene was extracted from tomato waste of paste processing line by classic solvent extraction (CSE) and ultrasound assisted extraction (UAE). Lycopene contents of samples were evaluated by spectrophotometric method. Results were validated by HPLC method. Experimental designs are evaluated and values are qualified by the statistical analysis program Minitab 15. For CSE and UAE %0,05 (w/v) BHT included hexane: acetone: ethanol (2:1:1) mixture was used as a solvent. While in CSE trials 50/1, 35/1 and 20/1 solvent/solid ratios, at 20^0C, 40^0C and 60^0C temperatures by 10, 20, 30 and 40 minutes runs applied, in UAE equipped by 24 kHz ultrasound probe, extraction trials 50/1, 35/1 and 20/1 solvent/solid ratios, 50, 65 and 90 W power at constant temperature 5^0C by 10, 20, 30 and 40 minutes runs applied. After extractions, polar and apolar layers were separated by means of centrifugal separator. The polar layer, containing lycopene was obtained and the absorbance was measured using a UV visible spectrophotometer at 503 nm and expressed as ppm using an extinction coefficient of $17.2x10^4$ $M^{-1}xcm^{-1}$. HPLC method is applied to see exact lycopene content and possible other carotenoids like β-carotene in extracts. For this method, methanol: acetonitril (90:10) mixture was used as a mobile phase at 0.9 ml/min flow rate. Applied detector was DAD at 475 nm. β-carotene and lycopene calibrations were done.

It was determined that the most efficient application for CSE is extraction by 50/1 solvent/solid ratio at 60^0C for 40 minutes run, for UAE is by 35/1 solvent/solid ratio, 90 W ultrasonic power for 30 minutes run. It states that ultrasonic applications are profitable than classic ones depending on time and temperature. On the other hand this study proves that spectrophotometric method is successful and has a perfect relation between HPLC methods to determine lycopene content of tomato derivatives.

Is Nutrigenomics the future of functional food?

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Functional foods are those that provide benefits beyond basic nutrition when consumed as part of the regular diet. There is no doubt that functional foods benefit to health, on the other hand human responses are variable. Nutrigenomics studies are providing understanding of the genetic basis of health and chronic disease as well as the interactions of genome with nutrition. Knowledge of gene-diet interactions provide chance for selecting foods leading to optimal health or reduced risk of chronic disease. Functional foods have the potential to provide a practical dietary solution to overcome a biochemical deficiency associated with variant genotype. The development of functional foods, guided by nutrigenomics research, offers a solution whereby people can continue to consume the foods they recognize and enjoy, which have had their nutritional content altered to better meet the needs of various identified subpopulations. Also with the knowledge of nutrigenomics, a pathogenic gene expression profile can be pursued by using mixtures of specific nutrients as nutraceuticals. Since nutrition can be employed in a natural way, it is expected that the general public will accept it more easily than drugs for the actual prevention of disease. In the pre-symptomatic phase expression profiles serving as early biomarkers as well as knowledge on the molecular pathogenesis will be of importance for designing and applying novel functional foods.

Functional properties of royal jelly, pollen, propolis and honey

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In this presentation the nutritional and functional properties of bee products and their health effects is reviewed as a summary of the scientific literature regarding the functional health enhancing effects of four bee products.

Apitherapy or "bee therapy" is the medicinal use of products made by honeybees. The honeybee products honey, pollen, royal jelly and propolis have been used by humans for thousands of years. Now the bio-active principles of these products are now understood.

While in early times there was no separation between food and medicine, nowadays, according to the food and medical laws in advanced industrial countries these two are now regarded as separate. In this logic honey (H) and pollen (P) are regarded as food while royal jelly (RJ) and propolis (Pr) are regarded as food supplements and not as medicine. According to the EU legislation medicinal claims for food products are not allowed, however specific health claims are possible if there is enough scientific evidence.

H, RJ, P and Pr have following common functional properties: antibacterial, antifungal, antiviral, antioxidant, anticancerogenic anti-inflammatory and immunomodulating. Honey has prebiotic, anti-osteoporisis and fertility-increasing effects. RJ has activity against fatigue, stress, hypoxia, high blood pressure, activates and protects the central nervous system. Pollen has positive nutritional effects and has detoxicating and chemopreventive properties. Propolis has hepatoprotective, detoxicating, muscle-relaxating and local anaesthetic effects.

Based on the above enumerated health enhancing properties it becomes evident that these four bee products can be regarded as functional food for which specific health claims can be made.

A functional product; yoghurt with *Saccharomyces boulardii*

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Sacchoromyces boulardii is a non pathogenic yeast which is used in the prevention and treatment of the diarrhoea depending on different etiologies. It is notified that *S. boulardii* prevents the intestinal problems caused by antibiotic usage and toxicity related to *Clostridium difficile.*

S. boulardii, because of being a yeast, is resistant to the antibiotics unlike other bacteria origined probiotics. Also it is not affected by gastric acid and bile. Therefore usage of it as a probiotic is more convenient than the other probiotic bacteria.

In this study it was intended to examine whether *S. boulardii* and yoghurt bacteria work together in the working medium. Also, the specifications of yoghurt samples determined and *S. boulardii*'s antagonistic effects examined.

Yoghurt production was performed with milk alone and milk including 1 % galactose. In addition to the yoghurt culture *S. boulardii* was added to the milk in the ratios of 2%, 4% and 6% to produce yoghurt. Control yoghurts were not include *S. boulardii.*

S. boulardii's antagonistic effect to *E.coli, Salmonella enteritisit, S. aureus* and *S. typhy* was determined and its relation with pH was examined. The antagonistic effect of *S. boulardii* to the pathogens was determined by well diffusion assay. According to the method no zone was observed on the agar plates.

The chemical characteristics of the yoghurt were determined. Adding galactose to milk was increased total solid but the ratio of total solid was not effected during storage time. The pH values showed a fluctuation. Lactic acid bacteria counts were high in the product including galactose and *S.boulardii.*
Lactic acid and citric acid in organic acids, were determined in the yoghurts by HPLC method.

Physical, nutritional and sensory evaluation of a novel extrusion-cooked lentils analog

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Abstract

The main objective of this research was to utilize extrusion processing for developing a wheat- and soy-based, low cost, nutritious, value-added 'lentil analog' product. The physical, chemical, nutritional characteristics and consumer acceptability of this product were also studied. While keeping the process conditions constant, lentil analog products using six formulations were produced using a pilot-scale twin screw extruder, in order to study the effect of ratio of defatted soy flour to wheat flour (50:50, 60:40 and 70:30) and various additives (monoglycerides, methyl cellulose gum and soy lecithin). Texture profile analysis of lentil analogs prepared for consumption revealed that the products formulated with 60:40 and 70:30 soy: wheat ratios exhibited a significantly higher hardness, adhesiveness and lower springiness as compared to all other extruded treatments. Descriptive sensory analysis of prepared products showed that all extruded treatments were similar to each other, but different from the natural lentil control. Treatment with no additives and soy: wheat ratios of 50:50, 60:40 and 70:30 were higher in descriptive attributes of beany flavor, particles/residuals and firmness, which were also seen in the control lentil sample. Consumer acceptability testing indicated that there were no significant differences ($p<0.05$) in acceptability among the selected treatments and the lentil control sample. Such lentil analog products can provide a low-cost, high-protein diet, which is also rich in essential vitamin and minerals, to the general population in countries of South Asia and the Middle-East, as a substitute for regular lentils that is increasingly a costly commodity.

Introduction

Among the commonly consumed food legumes, lentils occupy an important place in human nutrition in the world especially in the developing countries. Lentils are excellent sources of protein, carbohydrate, and dietary fiber (Khan et al. 1987; Sharma et al. 1989; Bahatty, 1988; Achinewhu and Akah, 2003). However, mature lentils seeds contain appreciable amounts of oligosaccharides of the raffinose family, such as raffinose, stachyose, manninotriose, and verbascose. Most researchers ascribe flatulence to the action of anaerobic intestinal microflora on these oligosaccharides, which cannot be degraded by mammalian digestive enzymes. Flatulence implies an obvious discomfort and is a major impediment to greater consumption of lentils (Dhindsa et al. 1985). Also, raw legume seeds have antinutritional substances such as trypsin inhibitors and hemagglutinins (Hernandez et al. 1998) which must be inactivated before they can be safely consumed.

Lentils are predominantly consumed in a thick soup made from the whole grain or split pulse. Total lentils production has remained constant during the past two decades, whereas the population has increased remarkably during the same period. As a result of this, the per capita availability of food legumes has significantly declined and the cost has raised (Solanki et al. 1999). Also, nutritional and cooking quality characteristics of food legumes are very important from a consumer point of view. Looking for a product similar for lentils contain no raffinose family, antinutritional substance with much shorter cooking time will be highly appreciated.

Extrusion cooking is widely used to manufacture carbohydrate- based foods. Extrusion conditions will be controlled to obtain optimized product quality. Extrusion cooking is essentially a process in which moistened starchy or proteinaceous foods are worked into a viscous, plastic like dough and cooked before being forced though a die (Riaz, 2000). Some results of cooking during the extrusion process are the gelatinization of starch, denaturation of protein, inactivation of many native enzymes causing food deterioration during storage, destruction of naturally occurring toxic substances, and diminishing of microbial counts in the final products (Colonna and Mercier, 1983). Extrusion cooking and puffing of cereals is widely practiced (Onwulata et al., 1998). Extrusion cooking produces a wide range of finished products from inexpensive raw materials with minimum processing time. Snacks are one of the fastest-growing segments of the food industry (İbanoğlu, et al., 2006). Consumers are buying snacks in greater variety and quantity than ever, and extrusion technology can produce almost all of them (Onwulata, et al., 2001).

Wheat grains are low in protein content (7-14%) and are deficient in certain amino acids such as lysine (Claughton and Pearce, 1989). Legumes such as soy bean on the other hand are higher in protein (18-24%) and can be used to support certain amino acids such as lysine (Potter, 1986, Rababah et al. 2006).

There is no information regarding the utilization of wheat and soy flour and in lentil analog production by extrusion processing. The objectives of the study are to study the extrusion parameters for producing lentil analog food using extrusion technology Also, to develop a value added extruded products that similar in its properties to the lentils in terms of nutritional, physical, and sensory properties and characterized by its ability to be consumed without causing any intestinal discomfort for the consumers using various formulas. In addition, to produce lentils analogue that has the ability to be prepared within very short period.

Materials and Methods

Materials

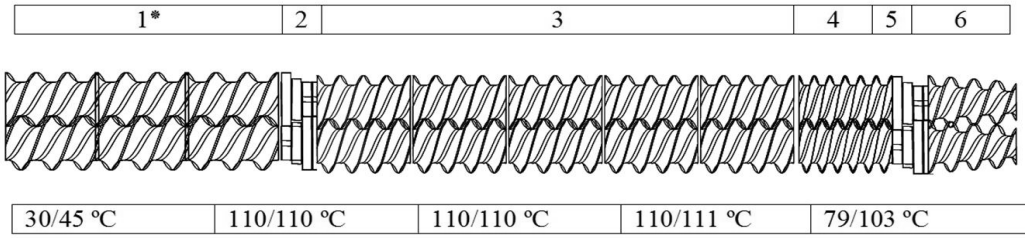
Soft Red winter whole wheat flour (Graham flour) obtained form cereal food processor (Kansas city), and the Soy flour obtained from (Honeysole,, USA).The following materials were generously donated by their respective company: Soy Lecithin (ADM, Decatur, IL); Methyl cellulose gum (Belcamp, MD); Bounus Blend multi-vitamins (Caravan ingredients, KS).

Formulations

Six different extruded treatments were formulated in order to study the effect of ratio of defatted Soy flour to Wheat flour in the presence of various additives. These treatments are: T1:50 %Soy + 50 %wheat and monoglyceride “SW(50:50)M”; T2: 50 %Soy + 50 %wheat and methyl cellulose gum “SW (50:50)G”; T3: 50 %Soy + 50 %wheat and lecithin “SW (50:50)L”; T4: 50 %Soy + 50 %wheat “SW (50:50)”; T5: 60 %Soy + 40 %wheat “SW (60:40)”; T6: 70 %Soy + 30 %wheat “SW (70:30)”. The amount of the protein, fat, fiber and ash in each formulation was approximated to be the same amount as the true lentil or higher.

Extrusion processing for lentil analog food

A pilot-scale twin screw extruder (model TX-52, Wenger Manufacturing, Inc., Sabetha, KS) with screw of medium-shear screw profile (Fig. 1) and circular die opening of 0.125" with 18 holes, was used to process all materials.



*Section Number	Number of the Parts	Desc Type
1	3	Full pitch of screw
2	1	Set(3) of forward kneading blocks
3	5	¾ pitch of screw
4	1	½ pitch of screw
5	1	Set (3) of forward kneading blocks
6	1	¾ pitch of core screw
	6 head	

Figure 1. The screw profile that we used for the lentil analog production and the temperature changes in the screw. It is in order from the inlet to the discharge.

Moisture content and water hydration capacity

Moisture content and Water Hydration Capacity (WHC) were determined on lentil analog products by using AACC official standard method (1999, 2008).

Color

The lightness of lentil analog extrudates was measured in triplicate using a Chroma Meter (Minolta CR-300, Minolta Corporation, Ramsey, NJ, USA) with a standard calibration plate CR-A44 with values of L^* =93.2, a^* =0.3139, and b^*=0.3204. The values were recorded and ten replicates were averaged.

Lentil analog texture

The lentil analog and the commercial lentil (control) were cooked according to a standard procedure before doing the texture analysis test and the sensory test. Texture of cooked lentil analog is most commonly found by using a compression test and measuring the strain. The strain force measurements, as well as other measurements, can be used to determine the hardness. A frequently used type of texture measurement is the texture profile analysis (TPA). Texture was determined with TA-XT2 Texture Analyzer (Texture Technologies Scarsdale, NY).

Sensory test

Quantitative descriptive analysis (QDA) and consumer acceptance test

The objectives of this evaluation were to profile six prototypes of extruded grain and compare them to a sample of lentils. Also, to select the three test samples closest to the control for consumer testing. Five-member highly trained descriptive panel profiled six samples of extruded grain and one sample of lentils. Products were prepared according to the method mentioned earlier. Consumer Sensory evaluation of lentil analog product was conducted to determine acceptability of the product.

Nutrition content

The nutritional content of the selected treatments (T3, T5, and T6 in addition to the control) which were selected after the quantitative descriptive analysis was determined. The approximate analysis include: moisture, fat, carbohydrate, fiber, ash, and protein. The approximate analysis was done at the Analytical Services Lab Animal, Kansas State University.

Differential Scanning Calorimeter (DSC)

A differential scanning calorimeter (DSC) (model Q100, TA Instruments, New Castle, DE) was used to determine the degree of starch gelatinization in the lentil analog produced by twin screw extruder using the method described by Alavi and others (2002).

Viscoamylograph

The visco/ amylograph is used to measure the viscosity changes of starch during pasting, shear thining and setback periods.

Statistics analysis

The analysis of variance and means comparison were conducted by the general linear model (Proc GLM) and Anova (Proc ANOVA) procedures with Statistical Analysis System software (version 8.2, SAS Institute, Inc., Cary, NC). Comparisons among treatments were analyzed by Tukey's Studentized Range (HSD), with a significance level at $P<0.05$.

Results and Discussion

Moisture content and water hydration capacity

There were insignificant differences in the moisture content among all the treatments. The moisture content of all the treatments ranged between 9.3-10.0%. This similarity in the moisture composition is primarily due to the similarity in the degree of water added to the system as well as due to the similarity in the main ingredients (wheat and soy flour) and the processing conditions. Also, the addition of the different additives such as the monoglyecrides, methylcellulose, and lecithin at 0.88% level did not affect significantly the moisture content in these treatments.

The ability of the lentil analog products to absorb water under certain conditions can be determined by the water hydration capacity (WHC). The Masoor (control) had significantly the lowest WHC compare to all other treatments. Also, there were insignificant differences at ($p<0.05$) in the WHC among T1-T4 while there was a significant difference between these treatments and the other treatments T5, T6, and the Massor. This variation could be due to the variation in the original composition between the Masoor and the other treatments as can be seen in Table 1. Therefore, it is expected that this variation in WHC will affect on all the physical, sensory properties of the lentil and the lentil analog products.

Table 1. Approximate analysis of the different selected treatments* in addition to the control

Treatments	%Protein	% Fat	%Fiber	% Ash
T3	$29.78^{c} \pm 0.03$	$0.74^{a} \pm 0.07$	$2.97^{b} \pm 0.03$	$2.56^{b} \pm 0.02$
T5	$32.68^{b} \pm 0.01$	$0.57^{b} \pm 0.02$	$2.76^{b} \pm 0.09$	$4.92^{a} \pm 0.01$
T6	$35.55^{a} \pm 0.2$	$0.51^{b} \pm 0.\ 0.05$	$2.96^{b} \pm 0.03$	$4.97^{a} \pm 0.02$
Masoor (control)	$24.83^{d} \pm 0.25$	$0.51^{b} \pm 0.05$	$3.05^{a} \pm 0.02$	$2.56^{b} \pm 0.03$

*T3:50 %Soy + 50 %wheat and lecithin "SW (50:50)L"; T4:50 %Soy + 50 %wheat "SW (50:50)"; T5: 60 %Soy + 40 %wheat "SW (60:40)"; T6: 70 %Soy + 30 %wheat "SW (70:30)"

Color

All the treatments were statistically the same for lightness, *L, a and b* values ($p<0.05$). This was expected because all of treatments were formed from the same type of the ingredients that might influence the color attributes and the variations in the composition did not make any significant differences in the color values.

Texture

Texture was affected by using different formulations and different additives in lentil analog productions. Hardness data showed that the Masoor (control) exhibited the highest value with statistically different

($p<0.05$) compare to all other treatments (Figure 2). This is expected due to the variation in the original composition between the Masoor and the other treatments which decreased the Masoor WHC. Also, it was found that the treatments containing higher amount of soy flour had significantly higher hardness values compare to all other treatments (Shogren et al. 2006; Singh et al. 2004), whereas the addition of the different additives such as the monoglyecrides, and methylcellulose at 0.88% level increased significantly the hardness values except for lecithin. This is could be due to the variation in the effect of these additives on the lentil analog composition and its physical properties.

Gumminess and chewiness data showed that the Masoor (control) had the highest value with statistically different ($p<0.05$) compare to all other treatments (Figure 2). This is expected since both of these physical parameters are related to the hardness values (Gumminess= cohesiveness*hardness, chewiness= gumminess*springiness).

It was found that the treatments containing higher amount of soy flour (60:40 and 70:30) were significantly higher in adhesiveness compared to all other treatments (Figure 4). The Masoor (control) had the lowest adhesiveness value with statistically different ($p<0.05$) compare to all other treatments (Figure 2). This could be due to the variation in the functionality of the different components in each formulation. However, there were no significant differences in the adhesiveness property among the treatments which include different emulsifiers (monoglyecrides, methylcellulose, and lecithin) in their formulations (Figure 2).

There were no significant differences in the cohesiveness property among all treatments. The Masoor and the other treatments that contain different additives (except for lecithin) exhibited a significantly higher springiness compared to the formula that contains soy: wheat flour (50:50).

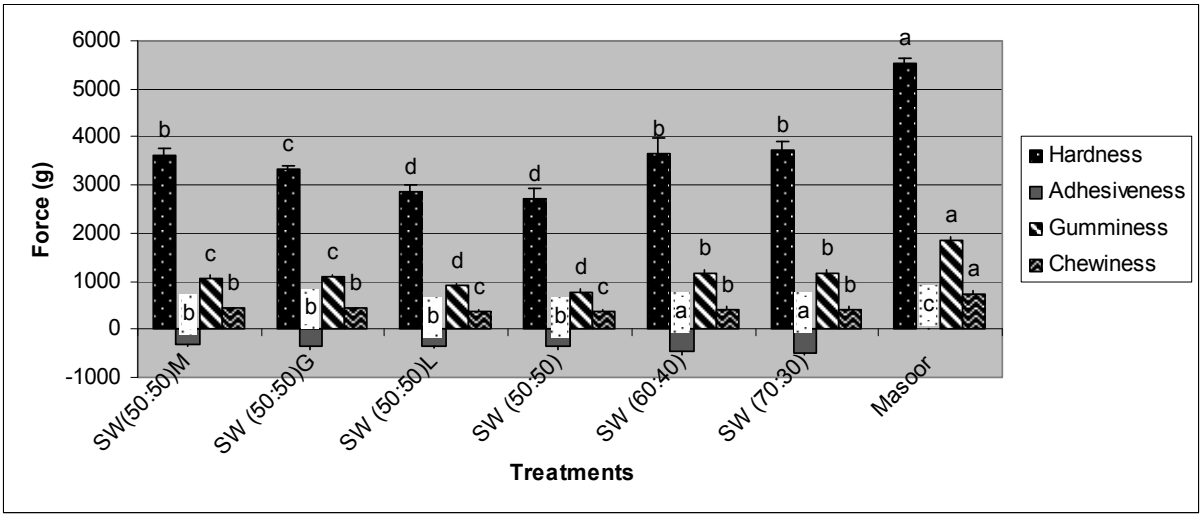


Figure 2. Comparison of hardness, adhesiveness, gumminess, and chewiness of lentil analog samples and the control (Massor) prepared under different treatments. Means with different superscripts indicate significant differences among all treatments ($p< 0.05$).

Quantitative descriptive analysis (QDA)

Flavor profiles show that test samples are similar to each other and different from the lentil control. The lentil control was mostly characterized by black pepper, beany, particles/residuals, and firmness attributes. Even though all samples are similar to each other and different from the control, it was recommended that the samples T3, T5, and T6 can be used further for consumer testing.

Consumer acceptance

Consumers found that the lentil analog formulated with 60: 40 and 70: 30 soy: wheat, and the Masoor (the control) were significantly more favorable than the lentil analog formulated with 50:50 soy: wheat (which

contains lecithin) in appearance, and flavor. The Masoor (control) exhibited the highest scores in overall acceptability, appearance, flavor, color, and mouthfeel but not with significant differences after using the Tukeys test for the mean separation.

Nutrition content

The nutritional content of the selected lentil analog products (T3, T5, T6, and the control) for protein, fat, fiber, and ash is shown in Table 1. The lentil analog formulated with 70: 30 soy: wheat (T6) had significantly the highest % protein content compare to all other treatments.

Degree of pregelatinization

Gelatinization temperature of T3, T5, T6 raw blends, and the control were observed at 71.68, 73.77, 73.94 and 74.85ºC, respectively. Peak was not found from extrudate samples in the range from 60 to 80ºC in which gelatinization temperature of T3, T5, T6, are present. It was concluded that all starch in dry blend are completely gelatinized by extrusion, and its %DG is 100% for all treatments as can be seen in the endotherms which were observed on all DSC thermograms for all extrudates at approximately 100°C.

Summary and Conclusions

- Texture profile analysis of lentil analogs prepared for consumption revealed that the Masoor (control) exhibited a significantly higher hardness, lowest adhesiveness as compared to all other extruded treatments.
- Descriptive sensory analysis of prepared products showed that all extruded treatments were similar to each other in terms of beany flavor, particles/residuals and firmness, but different from the natural lentil control.
- Treatment with soy: wheat ratios of 50:50 L, 60:40 and 70:30 were closest to the control in descriptive attributes of beany flavor, particles/residuals and firmness.
- Consumer acceptability testing (9-point hedonic scale) indicated that there were no significant differences ($p<0.05$) in acceptability among the selected treatments and the lentil control sample. However, further studies need to be done to improve the starchy mouth feel, particles integrity, beany and black pepper flavor.
- By using DSC, it was found that all starch in dry blend are completely gelatinized by extrusion, and its %DG is 100% for all treatments were observed on all DSC thermograms for all extrudates at approximately 100°C.

Acknowledgments

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Chemical and functional properties of full fat and defatted white melon (*Cucumeropsis mannii)* seed flours

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Developing nations do not produce enough food and of the right nutritional quality to meet daily needs of their ever growing population, therefore there is need to search for nutritious and locally available underutilized food products in order to effectively exploit all the potential sources of foods (Oshodi et al. 1999). *Cucumeropsis* m*annii* is an oil rich seed crop, a source of dietary proteins and contained nutritionally important amino acids (Ogunbusola et al, 2008). Its industrial applications however, depend on the knowledge of their protein quality and functional properties. The work is aimed at determining the proximate composition, antinutritional and functional properties of full fat and defatted *Cucumeropsis mannii* seed flours.

The seeds of *Cucumeropsis mannii* were bought from farmers in Omuo-Ekiti, Ekiti State, Nigeria. The seeds were shelled, washed, dried in a hot air oven at 50^0C, pulverized and sieved to pass through a 300µm sieve. A portion of the flour obtained was defatted continuously for 8hours using n-hexane while the other part was not defatted. The full fat and defatted seed flours were analyzed for proximate composition (AOAC, 2005), mineral analysis (IITA, 1980), antinutritional factors (AOAC, 2005; Harbone, 1993) and functional properties (Coffmann and Garcia, 1977; Fagbemi, 1999; Beuchat, 1977).

The results showed that the full fat and defatted seed flours contained the following in g/100g sample; 5.0 and 5.1, 45.8 and 1.0; 39.4 and 78.7; 3.45 and 4.40; 1.50 and 3.05; and, 4.85 and 7.75 for moisture, crude fat, protein, ash, crude fibre and carbohydrate respectively. The most abundant mineral elements in the seed flour (mg/100g) are potassium (198.5), Nickel (30.0) and magnesium (28.4). The water absorption capacity, oil absorption capacity, foaming capacity and stability, least gelation concentration, emulsion capacity and bulk density are 55.5 and 125.0%; 128.8 and 184.0%; 10.5 and 17.0%; 3.0 and 1.5%; 16.0 and 10.0g/ml, 85.0 and 115.0ml/g; and, 0.42 and 0.25g/ml respectively. Defatting significantly influenced the functional properties. The foaming capacity is sample concentration and pH dependent. The pH of minimum and maximum protein solubility of the seed flours are 3 – 4 and 11respectively. The anti nutritional composition of the seed flour revealed the following; Tannic acid, 1.54mg/100g; phytin phosphorus 0.70mg/g; phytic acid, 2.48mg/g; oxalate, 1.8mg/g; Alkaloids 1.97% and saponin 0.50%. This work has shown that *Cucumeropsis mannii* seed flour is a potential source of dietary oil, proteins and mineral elements for use in food formulation. Its functional properties suggest that it has potential for use as functional ingredients in soups and frozen desserts. The low antinutritional composition of the seed flour may further enhance its utilization.

Latest Trends in Sustainable Packaging

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It's a well-known fact that packaging plays a key role in differentiating the products: in triggering impulse purchases, in enabling ice cream's ritual, new occasions or trading down offers, or in prompting premium-product messages.

Sustainability in packaging mainly consists of finding the right balance between brand proposition, product safety, manufacturing, costs and environmental impact, as the environment is increasingly influencing consumers' choices and brand strategies.

Packaging design choices will influence the environment on the baseline of waste, green house gases and sustainable sourcing. The overall objective is to minimise packaging ending up in landfills as well as our greenhouse impact by choosing the right materials at the design stage, while maximising our use of renewable materials.

Moreover, Unilever is one of the key actors in the industry such as: 2 billion consumers use a Unilever product on any day, more than 170 countries in which our products are sold, 264 manufacturing sites worldwide, €89 million invested in community programmes worldwide and more than 163,000 employees. Therefore the mission of big industries such as Unilever play a key role in the environmental footprint whereas our goal is to double the size of the business whilst at the same time halving our environmental footprint. This encompasses the whole value chain – from the sourcing of raw materials through to consumer use and disposal of our products.

In this study, Unilever Sustainability Living Plan and the key metrics will be evaluated as well as innovative packaging design examples, and latest trends which are followed up by the industries to reduce their environmental impacts in terms of packaging, such as recycling, reusing, renewing and new technologies under development while moving decades.

Aluminum migration to chicken stew in semi- rigid Al- based packaging after thermal processing and during storage and comparing with EU simulants

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The main way for recognizing packages is dividing them to hard, semi-rigid and rigid. The use of semi-rigid packaging for ready- to eat foods increases day by day.

The packages which used in this study belong to this kind of packaging that made from 2 main layer; Aluminum and polypropylene, a kind of plastics. For improving the function of these plastics during process, forming and even use of them as food packaging, different kind of additives such as antioxidants, stabilizers, lubricants, antistatic substances and so on are added to them. But these days the safety of these packages especially due to the migration of additives from them is in doubt.

Migration is studied in two aspects: Global or overall migration which is the amount of substances transfer from packages to food (include recognized and nonrecognized substances). According to EU if the overall migration is lower than 10 mg/dm^2 or 60 mg/kg of food, the package will not have safety concern. But the migrated substances may be very harmful. Therefor the overall migration cannot assure the safety of packages by itself.

Specific migration is the measuring the transfer of certain and recognized substance from packages to food. Measuring the overall migration is only possible in stimulants. Ethanol 10%, acetic acid 3& and extra deionized water as stimulants were used for this purpose. Packages in 6 replications were filled with stimulants and got sterile. After that the overall migration were measured with gravimetric method. For all 3 simulants the amount of migration were lower than EU limit.

In this study since the base of these packages is Aluminum and it is considered as the element responsible for neurotic diseases especially Alzheimer, so it is chosen as specific migration substance. As the pH level of stew is between 2 EU stimulants, In this work distilled water and 3% acetic acid as stimulants, were processed exactly the same as chicken stews. The migration of aluminum in stew and stimulants was determined right after thermal process and during storage at 45°C by ICP_OES. Also the Al content in chicken stew before sterilization was measured.

The result showed that aluminum migration in water was very low and the migration in chicken stew was more than stimulants. The effect of time in amount of migration was very pronounced. Regarding the suggested provisional tolerable daily intake of 1 mg Al/kg body weight per day of the FAO/WHO Expert Committee on Food Additives, Al leaching from semi- rigid Al- based packages may add low doses of Al into diet but the determined amount for stored packages may be risky for children and teenagers.

Film forming properties of mammalian gelatins as biodegradable films

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Abstract

Gelatin from both bovine and porcine skin was used to manufacture films from solutions of varying gelatin concentrations using a casting approach. Mechanical properties, water vapor permeability (WVP) and oil permeability (OP) of all manufactured films were studied. No significant differences in tensile strength (TS) for different sources of gelatin were observed. However, TS values significantly increased for films manufactured from both gelatin sources when the concentration increased from 4% to 6%. No significant differences were observed either for puncture strength (PS) or elongation (E) for films manufactured from the different gelatin sources, with the exception being determined at an 8% concentration. Films manufactured from porcine gelatin had significantly lower WVP than bovine equivalents at a 4% gelatin concentration. The WVP of films manufactured from porcine gelatin significantly increased as gelatin concentration increased. OP showed no such significant differences when gelatin concentrations increased and was unaffected by gelatin source. The results of this study showed that films manufactured using higher concentrations of gelatin possessed good mechanical properties accompanied with low water and oil barrier properties.

Introduction

Food packaging is the largest growing sector within the plastic packaging market (Comstock et al. 2004). However, issues pertaining to sustainability, in light of dwindling energy reserves, pose challenges to the conventionally manufactured plastic-based packaging materials currently on the market today. Alternatively, sustainably sourced biopolymers have the potential to reduce or replace conventionally produced plastics which are consumed industrially, particularly for food packaging applications. Natural biopolymers have the advantages of being renewable in many cases, biodegradable or compostable in nature and where necessary, edible. Some examples of biopolymers that can form polymeric films are derived from polysaccharides (starch, chitosan/chitin, pectin, carrageenans etc.) and proteins (collagen, gelatin, whey, soy, corn etc.)

Among these materials, gelatin is an interesting material because dehydrated gelatin is partially a crystalline polymer and has a relatively low melting point (Sobral & Habitante, 2001; Park et al., 2008). Gelatin is an animal protein obtained by a controlled hydrolysis of the fibrous insoluble collagen present in the bones and skin generated as waste during animal slaughtering and processing (Patil et al. 2000; Martucci and Ruseckaite, 2010). Thus, it offers relatively low cost and possesses excellent functional and filmogenic properties (Eastoe and Leach, 1997; Arvanitoyannis et al., 1997a, Arvanitoyannis et al., 1997b, Cao et al., 2007; Rivero et al., 2009).

The effect of plasticizer on the thermal and functional properties of gelatin had been studied (Sobral et al., 2001), but less attention has focussed on the effect of utilising various ingredient concentrations and their subsequent impact on the properties of such films. Therefore, the objective of this study was to evaluate the mechanical properties, water vapor permeability and oil permeability properties of gelatin films manufactured using various ingredient concentrations from bovine and porcine sources.

Materials and Methods

Materials

Bovine skin and porcine skin gelatins were purchased from Sigma Aldrich (MO, USA) and Redbrook Ingredient Services (Dublin, Ireland). Glycerol used as plasticizer was purchased from Sigma Aldrich (MO, USA).

Methods

Gelatin films from bovine skin and porcine skin were prepared by solution casting employing concentrations which ranged from 4% to 8%. Glycerol was added as plasticizer and solutions were stirred using magnetic stirrer hotplate and heated to 80°C for 30 minutes. Films were cast by pouring pre-heated solutions onto level circular plates and dried for 24 hours at 50 ± 5% RH and 23 ± 2°C. After drying, films were peeled off from the plates and cut into test specimens.

Film thickness

Film thickness was measured with a hand-held digital micrometer (51031 Käfer, Villingen-Schwenningen, Germany). Measurements were made at different locations and the mean thicknesses were used to calculate the permeability of the films.

Measurement of mechanical properties

Mechanical properties of films; tensile strength (TS), elongation at break (E) and puncture strength of films were determined using Imperial 2500 instruments, mecmesin force and torque test solutions (Mecmesin Ltd., Slinfold, West Sussex, England) according to the ASTM-D882 (ASTM, 1985).

Measurement of water vapor permeability (WVP)

WVP of gelatin films were measured according to the WVP correction method of McHugh et al. (1993) which is a modification of the ASTM E-96 standard method (ASTM, 1990) for determining WVP of synthetic packaging materials. A volume of 6 ml of distilled water was added into each test circular cup and the film sample was tightly clamped over the cup opening. The cups were maintained under controlled temperature and humidity (50 ± 5% RH and 23 ± 2°C).

Measurement of oil permeability

Oil permeability of gelatin films was determined using the method of Wang et al. (2007). Whatman filter papers were held in a controlled temperature/humidity room (50 ± 5% RH and 23 ± 2°C) to establish a constant weight. A trimmed test film disc was placed on an individual filter paper and 5 ml of corn oil was spread onto the film surface and the test film was held for 24 hours. The oil and test film were removed and the filter paper reweighed.

Statistical analysis

The statistical analysis of the data was carried out by one-way analysis of the variance (ANOVA) and the LSD test (least significant difference) which showed the values statistically different. A significance level of $P < 0.05$ was used with Statgraphics Centurion XV software programme (StatPoint Inc., 2005).

Results and Discussion

Mechanical properties

The mechanical properties of gelatin films are presented in Table 1. The TS of bovine and porcine gelatin-based films were not significantly different from each other. This was also observed regardless of the gelatin concentrations used. However, TS values for films, manufactured from both sources of gelatin used in this study, significantly ($P < 0.05$) increased when the concentration increased from 4% to 6%. No further significant increases occurred when gelatin concentration increased from 6% to 8%.

Meanwhile, PS was unaffected by gelatin source. However, at an 8% gelatin concentration, PS for films manufactured using porcine-derived gelatin presented greater ($P < 0.05$) resistance to puncture than films manufactured from bovine-derived gelatin. This also happened to be the film with the greatest PS (20.42 N) of all films tested. The concentration of gelatin used also significantly ($P < 0.05$) affected PS, whereby the higher the concentration of gelatin used, the greater was PS.

No significant differences were observed for E values from films manufactured from both sources of gelatin and at gelatin concentrations between 4% and 6%. However, at an 8% gelatin concentration, E values for films derived from bovine-sourced gelatin had greater ($P < 0.05$) E than film equivalents manufactured from porcine-derived gelatin. However, when gelatin concentrations ranged from 4% to 6%, E properties decreased ($P < 0.05$), irrespective of gelatin species origin.

Table 1. Mechanical properties of bovine and porcine gelatin films

Samples	Thickness (mm)	Tensile strength (MPa)	Puncture strength (N)	Elongation (%)
4%				
Bovine skin	0.051 ± 0.003^{abc}	2.10 ± 0.72^{c}	8.98 ± 0.30^{d}	50.71 ± 4.83^{a}
Porcine skin	0.042 ± 0.014^{c}	$2.98 \pm 0.55d^{bc}$	8.97 ± 0.70^{d}	47.22 ± 4.04^{a}
6%				
Bovine skin	0.053 ± 0.007^{ab}	4.20 ± 0.05^{ab}	16.99 ± 0.98^{c}	3.36 ± 0.03^{b}
Porcine skin	0.050 ± 0.004^{bc}	4.76 ± 1.07^{a}	18.08 ± 1.13^{bc}	7.36 ± 1.52^{b}
8%				
Bovine skin	0.053 ± 0.005^{ab}	4.22 ± 0.21^{ab}	18.90 ± 0.97^{b}	45.19 ± 3.04^{a}
Porcine skin	0.059 ± 0.007^{a}	5.07 ± 0.34^{a}	20.42 ± 4.61^{a}	3.76 ± 0.47^{b}

Means in the same column followed by the same letter are not significantly difference ($P < 0.05$)

Water vapor permeability (WVP)

WVP values are indicated in Figure 1. Bovine and porcine gelatin behaved differently from each other in relation to WVP. Using a gelatin concentration of 4%, porcine-derived gelatin-based films had lower ($P < 0.05$) WVP values when compared to bovine-derived gelatin film equivalents. However, WVP values for both bovine and porcine-derived gelatin films were not significantly different from each other when 6% and 8% gelatin concentrations were used to manufacture films. On assessing the specific effect of concentration, films manufactured from bovine-derived gelatin showed no significant differences from each other with respect to WVP as the concentration of gelatin used to produce the films increased. However, films manufactured from porcine-derived gelatin showed increased ($P < 0.05$) resistance to WVP as the gelatin concentration increased from 4% to 6%, but not at any other concentration used.

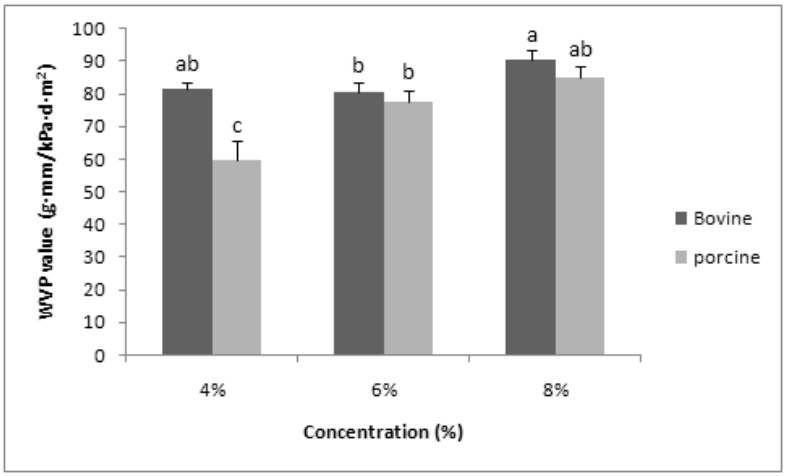

Figure 1. WVP test of bovine and porcine gelatin films

Overall, porcine skin with 4% concentration possessed the lowest WVP value, below 60 g·mm/kPa·d·m^2. In this study, it was noted that as the gelatin concentration increased in films, so too did the requirement

for greater plasticizer addition. This consequently led to an increase in WVP, but this was especially apparent for films manufactured from porcine-derived gelatin. It is well recognized that the addition of plasticizer to a protein-based film mixed solution causes the protein network to become less dense and more permeable (Cuq et al., 1997), however, the plasticizing effect noted in this study is interesting in that it appears to affect the same protein source in different ways, depending on the differences associated with the protein based on its species origin.

Oil permeability

As shown in Figure 2, OP of films manufactured from bovine- and porcine-derived gelatin and used at a 4% concentration showed no significant differences. However, for films manufactured using both 6% and 8% gelatin it was shown that there were significant ($P < 0.05$) differences in oil uptake by both films which resulted in differences in weight gain, with films manufactured from porcine-derived gelatin being lower or more resistant to oil uptake. In terms of gelatin concentration, no significant differences were observed within each species-defined film type with respect to OP.

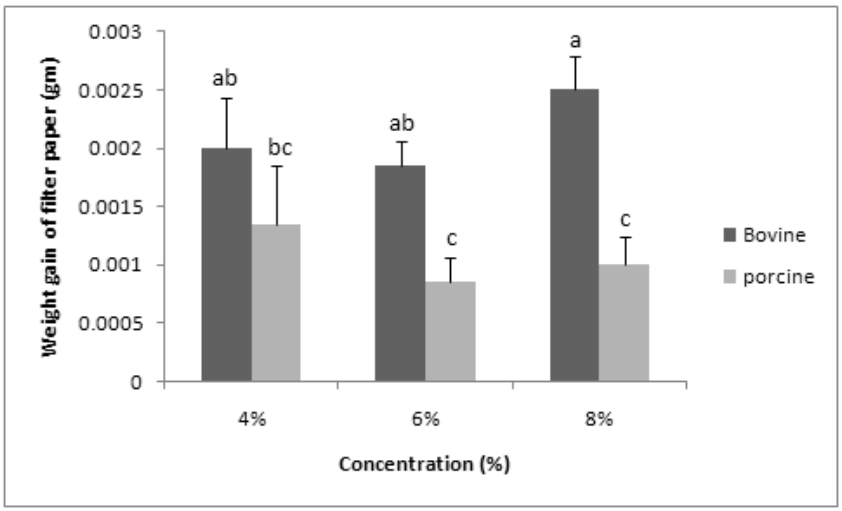

Figure 2. Oil permeability test of bovine and porcine gelatin films

Conclusions

Overall, it was shown in this study that gelatin formed very functional films which possessed some very good mechanical and barrier properties. Film properties, as affected by ingredient concentration, were interesting, but more so were the differences that emerged when gelatin was utilized from different animal sources for film manufacture and compared.

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Methods of packing and packaging commercially-produced beef burger

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Abstract

The objectives of this study were to study the quality packet and packaging. Samples of seven beef burger processing plants.

All samples were subjected to different quality control tests namely, Appearances of beef burger packaging included types of outer and inter of package materials packaging closing, system packaged the burger in the package-included number of the patties per package; method of patties put in the package, type of separation materials between burger patties, labeling on the package, date of production, expire date, content of burger patties.

The results indicate the following:- 1- Either carton packaged all samples or plastic materials, only sample (1) used inner plastic materials package, sample(2) used inner carton packaged but other samples not use inner packaging, finally closed by plastic tap, thermal pressing. 2- Samples within package were separated by plastic or waxed paper, or a tracing paper not wax as in sample (4), number of burger per package ranged from (8) to (10). 3- Date of production was fixed on all samples with the exception of one sample. The expiry dates ranged between (3-6) months and doesn't fix on sample only; contents of burger patties doesn't but in the package on one sample only.

In conclusion there was difference between beef burger processing in Baghdad City, plants to use a package are not following the Iraqi standard rules, which in turn indicated the importance of ministry quality control routine checks to protective the production commercially from pollution.

Introduction

The manufacturing of meat and its products emerged technically and technologically in the seventies of the past century. The growth and the development of this manufacture encouraged the increasing demand on the processes meat products and the products ready-to-consumption and that what has been confirmed by Cross et al (1980), as he considers beef burger one of the widely spread products and the most demanded by the consumer. So, researchers and specialized persons paid a great attention to developing the quality of the processed burger, from one hand, and decreasing the cost of this product from the other hand (Vaisy, 1975). The wide spread of beef burger production resulted in the emergence of several qualities in beef burger due to the variation in the packs and packaging. So, it was obligatory to use certain type of packing and packaging to preserve burger from contamination and that surely requires certain system of packaging to protect the burger from damage and that was confirmed by the Iraqi Standard Specification No. (1580) in (1990) issued by the Central System of Standardization and Quality Control, which recommends the necessity to package burger in plastic, cellophane or cartoon packs which are approved by health authorities and these packs should be tightly sealed because manufacturing beef burger involves the interference of many factors that influence its quality because of contents of humidity, protein, fats and other ingredients, the Saudi standard specification No. (1) in 1973 requires the necessity of mentioning the ingredients on the cover of the pack so that the consumer can know about the ingredients. Also, the Saudi standard specification No. (457) in 1986 necessitates the mention of the expiry date on the cover of the beef burger pack and it should not exceed (9) months from the date of production. The are other factors which give a clear indication to the processed beef burger including manufacturing conditions, circulation, reserving and storing methods, packaging method and the type of packs used.

The difficult circumstances Iraq has been passing through led to using poor types of packs and these circumstances weakened the standard control systems concerning food products. So, this study conducted a series of tests relevant to packing, packaging and the indications on the packs of the beef burger produced in Baghdad commercially, and as follows:

1. The Identify the extent to which packing, packaging, covering and the indications on the cover meet the financial regulations.
2. Evaluating the homogeneity of quality specifications of the processed meat.
3. The role of packing and packaging in protecting the beef burger quality and reserving it from contamination.

Materials and Methods

Designing the experiment

The experiment involved conducting quality control tests in relevance with packing, packaging, and the indications on the cover of the burger packs, which is commercially produced in seven main factories in Baghdad, and as shown in Figure (1).

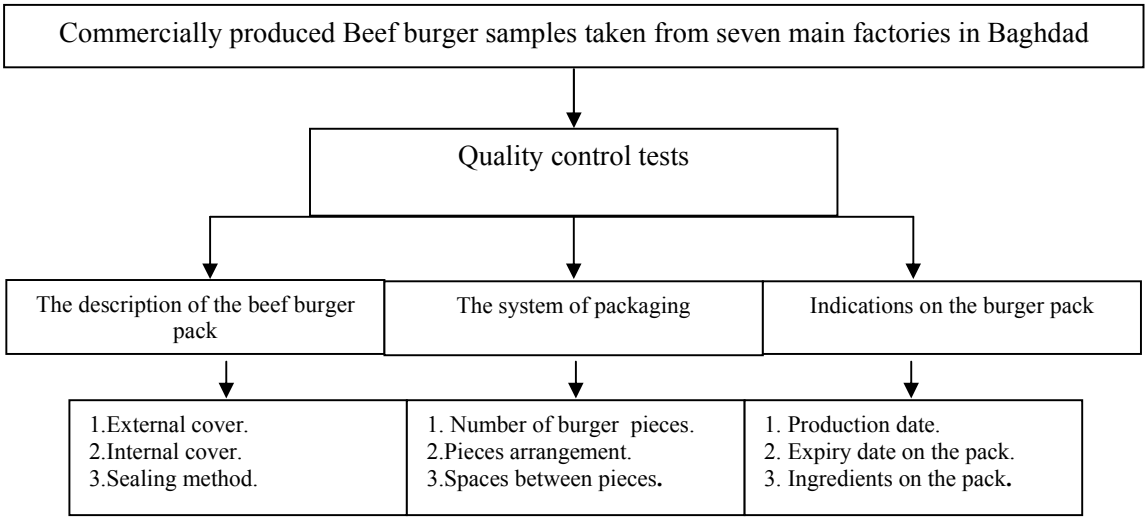

Figure 1. The diagram of the experiment design

Samples collecting methods

Four complete beef burger packs manufactured in seven main factories in Baghdad market packs were purchased. All the sample packs were included for the purposes of full description of the packaging system, packing and the indications shown on the packs covers. Bellow, are the names of the factories from which these samples were taken, and as shown in Table (1).

Table 1. Names of the factories commercially producing beef burger

Factory Number	The commercial name
1	Al-Firdaws
2	Latheeth
3	Al-Salem
4	Nabeel
5	Abu-Michael
6	Al-Wataneyyah
7	Abu-Yunan

The description of packaging system of the beef burger

The description of the internal and the external of the pack and the sealing of the pack is as determined by the Iraqi Standard Specification No. (1580) in 1990 issued by the central system for Standardization and quality control.

Burger packing system in the pack

The number of the burger pieces in each pack, the number and the spaces between burger pieces was all shown.

Indications on the cover of the beef burger pack

These included the date of production, expiry date and the beef burger ingredients according to Iraqi standard specification No. (1580) in 1990 issued by the Central System of Standardization and Quality Control and the Saudi standard specification No. (457) in 1986 issued by The Arab Saudi Authority for Specifications and Standards.

Results and Discussion

Description of the pack

A good attention should be paid to the pack of the commercially-produced beef burger because the beef burger is considered a good medium for the growth of organisms as it includes the suitable elements necessary for the growth of these organisms. These elements includes high moisture, Nitrogen and Carbohydrate matters and the PH of the meat is considered a good medium for the growth of these organisms. The process contributes in spreading the organisms that exist on the surfaces of meat pieces through the process of chopping and mincing in addition to other factors such as temperature and the period of storing. So, packing plays a great role in preserving the quality of the beef burger and minimizing its contamination and damage (Al-Taee, 1995). The good-sealed burger packs are considered very important in preserving the beef burger by maintaining the same level of moisture (preventing evaporation), because moisture is considered a vital factor in determining the good quality of meat products (Al-Aswad, 2000). Also, the packs play a great role in preserving the protein included in the beef burger, which is characterized with a high nutritious value as it involves the important amino acids which can not be produced by the human body (USMEF, 2005). Packs are also regarded as highly important in protecting fats from oxidation (Willenberg, 2003) because the oxidation of fats results in the damage of meat products (Gray et al, 1996) which is caused by the exposure of the beef burger to air and that in turn leads to the increase of free fat acids and the oxidation of the unsaturated bonds and that in turn results in undesirable odor and bad taste as well as being responsible for the changes in the burger quality (Gandermer, 2002). Poor packing also leads to decomposition which leads to the increase of Amines and carboxyl groups as a result of the decomposition of the protein in the beef burger. The quality of the burger deteriorates when ammonia compounds are produced, which are regarded an evidence of decomposition development (Al-Aswad, 2000). The method of packing and packaging influences the color of the burger, which is considered a distinguishing feature by which the consumer can tell about the good or the bad quality of the beef burger (Renerre, 1990) and which consequently reflects on marketing the product (Clydestale, 1998).

Table 2. shows the description of the packing system of a sample of beef burger. It is clear that the outer cover of the pack is made of cardboard or plastic for all the packs, while in the inner part plastic was used for the first sample and a cardboard for the second sample, but this was not available for the rest of the samples although that is so important in preserving the required standard specifications of the burger. Chu et al (1988) emphasized that meat products should be packed in air-evacuated sacks in order that these products will not influenced by oxygen, because oxygen and the PH are the main factors which are responsible for the change in color of the meat and its products and that is due to the myoglobin changing into oxi-myoglobin by the increase of oxygen permeability to the meat (Ledward et al, 1971), Cornforth et al, 1985, Chu et al 1988). Al-Taee (2004) found that most of the factories producing beef burger in

Baghdad commercially do not use the internal cover for packing and they used only a metal stapler to seal the sac. While for the sample of this study an adhesive tape was used to seal the cardboard packs as in the first sample and the method of thermal sealing of the plastic containers was used for the rest of the samples. Moreover, it is necessary that sealing the packs should be tight so that the product would not be exposed to undesirable external circumstances which cause changes and damages in quality and health.

Table 2. The description of packaging system of the commercially-produced beef burger

Factory number	Outer cover	Internal cover	Sealing method
1	Cardboard cover	Plastic sac	Adhesive transparent tape
2	Plastic sac	Cardboard cover	Thermally sealed
3	Plastic sac	No cover	Thermally sealed
4	Plastic sac	No cover	Thermally sealed
5	Plastic sac	No cover	Thermally sealed
6	Plastic sac	No cover	Thermally sealed
7	Plastic sac	No cover	Thermally sealed

Beef burger packing system

Table 3. shows the arrangement of the burger pieces, number and the spaces between them inside the pack. It was found that the number of the burger pieces within one pack, in most of the samples, was (8) or (10) arranged as rows and each of these rows includes (4) or (5) pieces. The arrangement of the burger pieces in the pack has an impact on the quality of the beef burger through changing the size of the piece. Cross et al (1980) found that these is a significant effect of the piece size on the taste and cooking. Also, the size and diameter of the piece have great importance in conducting the external heat into the piece inner parts uniformly. Because using the waxy papers as a separating material between each two pieces to prevent their sticking with each other. And it was noticed that, in one sample only, a non-waxy transparent paper was used as a separating material between the burger pieces, while three other samples used plastic pieces for separating the beef burger pieces instead of the paper. Al-Taee (2004) stated that most of the factories which produce the beef burger commercially in Baghdad used the plastic pieces between the burger pieces while other factories didn't.

Table 3. Packing system inside the pack of the commercially-produced beef burger

Factory number	No. of pieces per pack	Arrangement of the burger pieces	Spaces between pieces
1	8	Rows: 4 pieces/row	Plastic piece
2	8	Rows: 4 pieces/row	Waxy paper
3	10	Rows: 5 pieces/row	Plastic piece
4	8	Rows: 4 pieces/row	Non-Waxy transparent paper
5	10	Rows: 5 pieces/row	Plastic piece
6	8	Rows: 4 pieces/row	Waxy paper
7	10	Rows: 5 pieces/row	Waxy paper

Indications on the pack cover

From table (4), it was found that the production date is written on the beef burger packs except for sample of factory (3). The Iraqi Standard Specifications stipulate that the production date should be permanently written on the pack clearly that it can not be omitted or scratched or changed as this date is very important in determining the expiry date of the product. From table (4), it is evident that there is a great variation in identifying the expiry date of the product ranging from the absence of a written date on the pack in the sample Number (5), (3) months for in the sample (7), four months for the sample (6), six months for the samples (1, 2, 3 and 4). This indicate that the expiry date was not determined on a technical or legislative bases and the Saudi Standard Specification No. (457) for the year 1986 stipulated that expiry date for the beef burger should not exceed nine months and mentioning the nutritious ingredients of the commercially-

produced beef burger without mentioning the ratios. In the table, it is clear that no ingredients mentioned in the burger produced by the sample number (5) although the Central System for Standardization and Quality Control stipulated that ingredients should be mentioned clearly and should never be covered with drawings or photos or veiled by covers as well as writing it in a way that it couldn't be removed from the cover and the list should be real and not forged and the additives to the beef burger components should be mentioned. In another study about the commercially-produced beef burger in Baghdad, it was shown that some factories didn't put the important indications on the packs in contrast to what is stipulated by the Iraqi Standard Specification (Al-Taee, 2004).

Table 4. Indications on the packs of the commercially-produced beef burger

Factory number	Production date	Expiry date	Ingredients indicated on the pack in sequence
1	Written on the pack	Six months	Beef + spices + salt
2	Written on the pack	Six months	Beef + spices + salt
3	No production date	Six months	Beef + soy bean protein bread powder + spices + salt
4	Written on the pack	Six months	Beef + flour + spices + bread powder
5	Written on the pack	No date	----
6	Written on the pack	Four months	Beef + spics + salt + bread powder + garlic + onions
7	Written on the pack	Three months	Beef + spices + salt

Conclusions and recommendations

Conclusions

A- It was clear that most of the factories don't use the internal cover in spite of its great importance in preserving the quality of the beef burger.
B- Most of the factories use the plastic pieces separating the burger pieces but one factory only uses non-waxy transparent paper.
C- It was notices that one factory did not write the important indications on the beef burger pack and this implies that this is a violation of heeding to the regulations concerning packing and packaging.

Recommendations

A- Conducting a health and quality study on the materials of packing used in packing and packaging the beef burger produced in Iraq.
B- Periodic and intensive Quality tests should be conducted by the competent authorities on the products of the relevant factories in order to develop and improve the quality of the beef burger produced.

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Characterization of self assembled silver nanocomposite as an active packaging

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Developing antimicrobial silver nanocomposites is one of the emerging research activities in the fields of polymer, applied microbiology, medical and food packaging. Active packaging, an innovative concept and new generation of food packaging, is a packaging based on deliberate interaction with food or food environment. The purpose of active packaging is not only to protect food quality but also to improve food properties and extend shelf life of food.

Silver nanoparticles with a size of about 5 nm were prepared by chemical reduction using polyethylene glycol (PEG) as reducing agent as well as stabilizer. Silver nanoparticles assembled onto low density polyethylene (LDPE) films by using the layer-by-layer (LBL) method. Nanocomposite films were built by sequential dipping of a LDPE film in either anionic colloid containing nanoparticles or cationic chitosan. Silver nanoparticles capped with polyethylene glycol (PEG) led to the formation of a colored film possessing antimicrobial properties with the thickness of 2, 4, 8, 12 and 20 bilayers. Silver nanocomposite films were characterized by atomic force microscopy (AFM) and UV-vis spectroscopy. Thermal, mechanical and antimicrobial properties of self assembled nanocomposite were investigated.

Atomic force micrograph of nanocomposites proved uniform distribution of nanoparticles on the surface of LDPE films. Differential scanning calorimetry (DSC) results showed that the deposition of silver nanoparticles increased the crystallinity of the composites. Deposition of silver nanoparticles influenced Young modulus of LDPE films significantly. Chitosan coating resulted in more flexibility and improved elongation strength significantly. Antimicrobial activity of silver nanocomposites against *Escherichia coli* ATCC 13706, *Staphylococcus aureus* ATCC12600 was evaluated by quantitative dynamic shake flask test. Growth kinetic parameters of *E.coli* and *S.aureus* affected by silver nanocomposites were calculated by modeling of absorbance data according to Gomperz equation. LDPE-silver nanocomposite resulted in increasing lag time and reducing maximum bacterial concentration significantly ($p<0.05$).

Deposition of silver nanoparticles and chitosan could render LDPE films as an active as well as antimicrobial food packaging application.

Production of single cell protein by *Saccharomyces cerevisiae* and *Candida utilis* from treated (dephenolized) and untreated olive mill waste

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Abstract

Olive mill waste (OMW) is a large by-product of olive oil processing with high BOD and COD value, and high phenolic content,with phytoxic properties and a problematic biodegradation process. Our main objective is the utilization of OMW for the production of single cell protein (SCP-supplement for human/animal nutrition), and concomitant reduction of BOD and COD. In our study, OMW was dephonolized using macroporous absorbing resins and utilized as fermentation medium for the cultivation of *Saccharomyces cerevisiae* and *Candida utilis* (sources of SCP). Dephenolized and untreated OMW with or without addition of nitrogen sources were as used as bioprocess medium. Also, cheese whey protein concentrate (WPC) was used as an additional nitrogen/nutrient source. The effects of total sugar concentration (condensed/ uncondensed OMW, addition of WPC), phenol content, type and concentration of nitrogen sources, process pH and temperature, inoculum concentration, agitation rate, and type of yeast on SCP production were examined. The results showed that *Candida utilis* exhibited somewhat better performance than *Saccharomyces cerevisiae* in most experiments (higher biomass). However, for each strain, different optimal conditions for growth were observed. Yeast extract and ammonium sulphate seemed to enhance biomass production more than other nitrogen sources, and DCW stimulated yeast growth. Condensing OMW at a 3:1 rate improved SCP production, as did a high agitation rate and 10% inoculum. Dephenolizing increased the production of SCP. Optimal biomass concentration reached or exceeded 10 g/l (pure protein concentration ~80%). Moreover, BOD and COD values decreased after dephonolization (~3 to 5-time reduction), and dropped further after fermentation and removal of biomass. Overall, OMW could be successfully utilized for SCP production and offer high added value. BOD and COD levels decreased significantly, and phenol content was almost zeroed, facilitating its biodegradation or potential use for irrigation.

Introduction

Olive mill waste (OMW) is a large by-product of olive oil processing with high BOD (~40.000 ppm) and COD (~50.000 ppm) value, and high phenolic content (~0,5%),with phytoxic properties and a problematic biodegradation process [1-2]. The aerobic degradation of OMW is partly blocked by the polyphenols found in OMW, and thus phenol-degrading microorganisms, mainly yeasts and fungi have been used in order to reduce phenol content of OMW and facilitate further biodegradation of the waste [2-5]. Also, another process involves the removal of polyphenols from OMW by alkaline treatment [5], which is however costly. In our approach, an ultrafiltration method was employed for concentrating and removing most of the polyphenols found in OMW (which can be used as a polyphenol concentrate/powder in food and pharmaceutical applications), in order to pave the way not only for the degradation ond reduction of BOD and COD of the waste, but also for the utilization of this substrate for the production of single cell protein. The latter has been produced from several yeast and fungi, such as *Candida*, *Schizosaccharomyces*, *Saccharomyces*, *Pleurotus*, *Aspergillus*, etc, but only on phenol-containing, untreated OMW [5-7]. In this study we examine the production of SCP from GRASS organisms, *Saccharomyces cerevisiae* and *Candida utilis* using dephenolized OMW.

Materials and Methods

Substrate and organisms: Olive mill waste was used as the basic substrate for the comparative production of single cell protein by *Candida utilis* and *Saccharomyces cerevisiae* (bakers' yeast), with or without the addition of organic and inorganic nitrogen sources, namely yeast extract, peptone, ammonium nitrate and

ammonium sulphate, and in some cases with the supplementation of whey protein concentrate (where whey proteins were isolated by ultrafiltration followed by thermal condensation) to increase nitrogen (and sugar-mineral) content of the fermentation medium. In most cases, dephenolized OMW was used. This was dephenolized (i.e. polyphenols were largely removed) by ultrafiltration using macroporous absorbing resins, and in some cases OMW was condensed by thermal treatment at 80°C to produce media concentrated by 2:1, 3:1, and 4:1 ratios.

Fermentation conditions: Agitation speed was either 250rpm, or 350 rpm, temperature was either 25°C or 30°C, initial process pH was either 5, 6, or 7, and inoculum was added at 5%, 10% and 15%. All experiments were carried out in 500ml shake flasks containing 250ml substrate, using an incubating rotary shaker. The pH was manually controlled by adding a buffer solution of 1 g/l of K2HPO4 and KH2PO4 solutions, and by manual adjustment (when/if necessary) after each sampling, by adding aseptically appropriate volumes of 1N NaOH or 1N HCl.

Analytical methods: Total biomass was assessed gravimetrically, by centrifugation of the fermentation broth at 5000rpm for 30 min, followed by drying of the precipitate at 105°C for at least 10h to constant weight.Total sugar content of the centrifugate of the process medium (after removal of biomass debri) was analysed by the dinitrosalycilic acid method. Ttotal proteins in the dry cell precipitate were assessed by the Bradford method, total phenols in the process medium by the Folin-Ciocalteu method. Respiratory Quotient (CO2/O2) was determined by measuring the CO2 produced by the culture and the available O2 in the flasks using an IR-gas analyser whose probe was inserted into the headspace of the fermentation flasks. The pH was measured by a bench pH-meter.

Results and Discussion

Effect of dephenolization

Figure 1 shows the effect of dephenolization on the production of biomass and sugar consumption by *S. cerevisiae*. Fermentation were carried out at 30°C, pH 5, and 250rpm agitation rate using a 5% inoculum. Biomass concentration increased from 0.45 g/l to 1.05 g/l in the dephenolized medium, while sugar consumption was higher by approximately 5g/l in the dephonolized OMW, although sugar utilization was incomplete in both treated (dephonlized) and untreated media. From an initial sugar concentration of approximately 22 g/l, untreated medium contained 14 g/l by the end of the fermentation, while dephenolized medium contained 10,4 g/l of residual sugars, showing that a limiting factor existed in both cases, preventing complete utilization of carbon sources. RW values were near zero for untreated OMW, showing very slow metabolism, while RQ was distinctively higher for the better-growing cultures in dephenolized OMW. Similar effects were observed with *Candida utilis.*

Effect of nitrogen sources

As OMW is poor in nitrogen, which is essential for biomass growth and SCP accumulation, and probably the limiting factor for sugar utilization, the effect of adding different organic and inorganic sources to OMW was studied. Namely, 5g/l of either yeast extract, peptone, ammonium nitrate and ammonium sulphate were added to the dephenolized process medium. Fermentation were carried out at 30°C, pH 5 and 250rpm agitation rate using a 5% inoculums. The effects on *S. cerevisiae* biomass concentration compared to dephenolized medium without any additives are shown in Fig. 2a. Ammonium sulphate seems to enhance biomass growth of *S. cerevisiae* significantly, much more than any other nitrogen source studied here, as it reached nearly 6,5 g/l after 80h. In contrast, *C. utilis* biomass reached maximum level (about 9 g/l at 70 h) after addition of yeast extract, which also offers sugars, minerals and vitamins to the yeast cells, apart from organic nitrogen (Fig.2b). The maximum average growth rate (until the time of peak biomass concentration) for *S. cerevisiae* was 0.0677 g/l/h with ammonium sulphate added, while for *C. utilis* the maximum average growth rate was 0.1325, thus showing a significantly faster growth rate for *C. utilis*. Sugar utilization was indeed improved by the addition of nitrogen sources, leading e.g. to a reduced 7 g/l of residual sugars in the process of *C. utilis* with added yeast extract (data not shown).

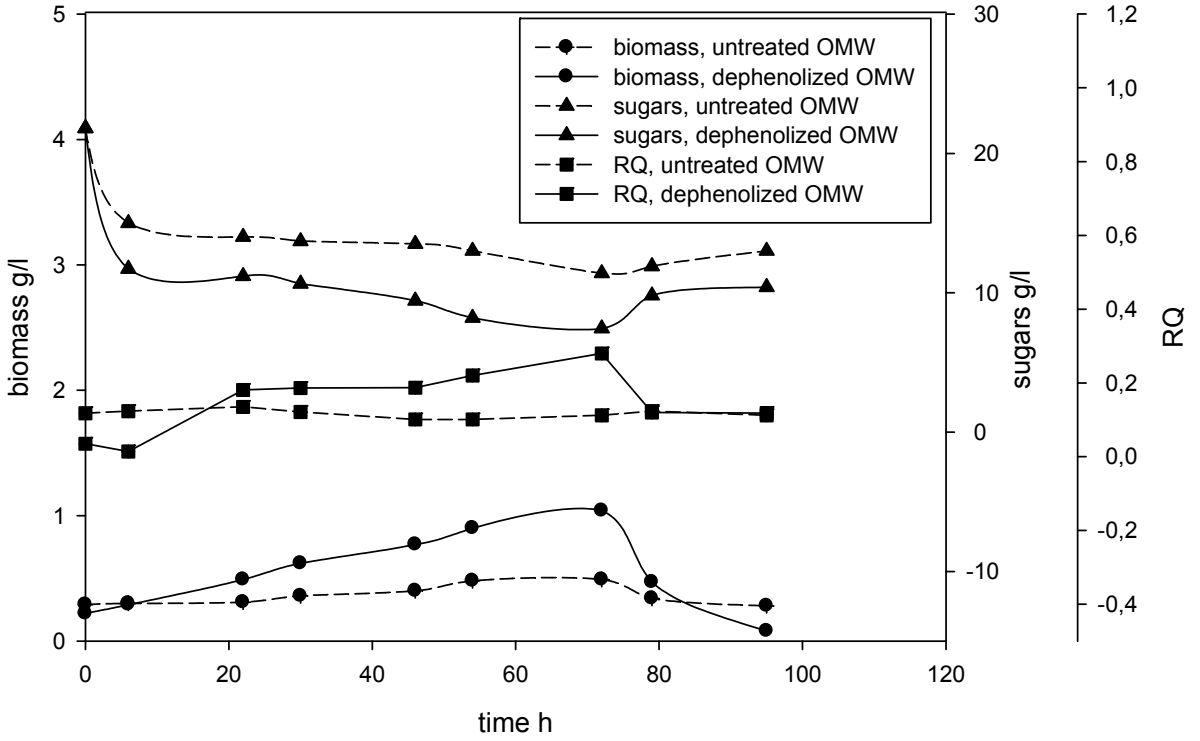
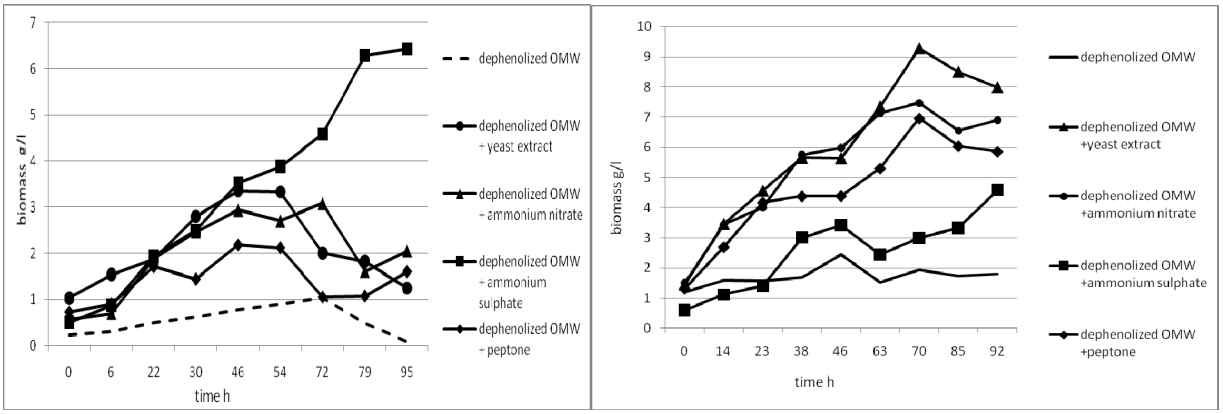


Figure1. Time profiles of biomass and sugar concentration during cultivation of *S. cerevisiae* in crude (untreated) and dephenolized (treated) OMW. Process conditions: temperature 30°C, 250rpm, 5% inoculum.

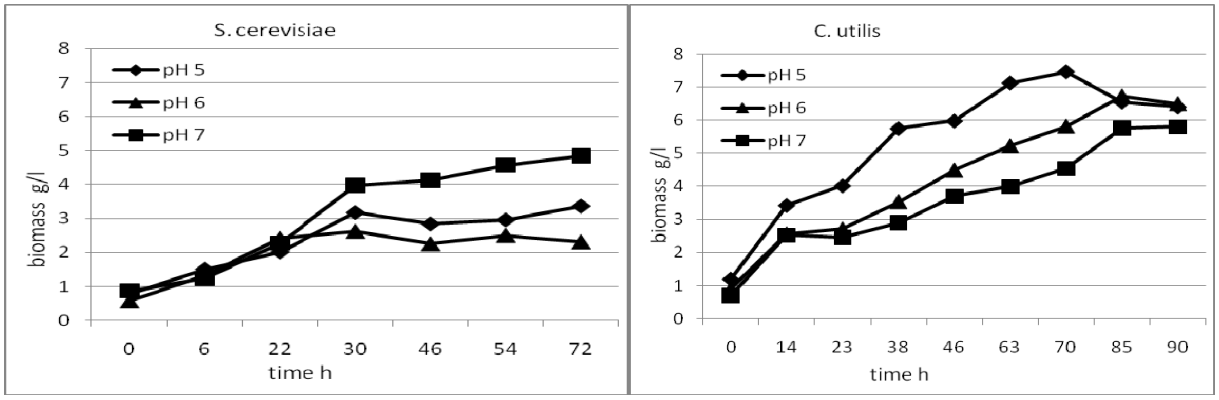


(a) (b)

Figure 2. Time profiles of (a) *S. cerevisiae* and (b) *C. utilis* biomass concentration in dephenolized OMW supplemented with organic and inorganic nitrogen sources at 5g/l concentration (compared to control without additional nitrogen process conditions: temperature 30°C, 250rpm, 5% inoculum.

Effect of pH

The pH during fermentation is a crucial parameter in yeast growth, and several optimal pH values exist for different yeasts. Here, optimal pH for growth of *S. cerevisiae* was pH 7, while *C. utilis* grew best at pH 5, where peak biomass concentration was 7,5 g/l (Fig. 3). Again, *C. utilis* reached a higher biomass level and had a higher growth rate compared to *S. cerevisiae*.



(a) (b)

Figure 3. Time profiles of (a) *S. cerevisiae* and (b) *C. utilis* biomass concentration at different process pH values in dephenolized OMW supplemented 5 g/l ammonium nitrate. Process conditions: temperature 30°C, 250rpm, 5% inoculum.

Effect of inoculum

The percentage of inoculum used in most cases was 5%, as it was found that it was optimal for *C. utilis* and that there was little difference (as to the final biomass levels) among an inoculum of 5%, 10% or 15% for *S. cerevisiae*. Also, the prior adaptation of both cultures to an OMW-based inoculum was essential for ensuring suffient growth of the cultures in the OMW-based process medium (data not shown).

Table 1. Phenol content of untreated, dephenolised, and condensed OMW (mean values of triplicate samples).

Medium	Phenol content (g/l)	Sugar content (g/l)
Untreated (crude) OMW	6,00	22,1
Dephenolized OMW	0,33	19,2
Dephenolized OMW, concentrated 2 times (2:1 concentration)	0,83	27,8
Dephenolized OMW, concentrated 4 times (3:1 concentration)	1,10	35,1
Dephenolized OMW, concentrated 4 times (4:1 concentration)	1,42	41,7

Effect of medium concentration condensation

The dephenolized OMW based medium was thermally condensed (evaporated) at 80°C in order to produce media with 2,3 and 4 times the sugar concentration of the initial medium and make available more carbohydrates to the yeast studied. At the same time all media were supplemented with 5 g/l yeast extract to cover any shortage of nitrogen or minerals sources. However, this condensation also increased the phenol content of the condensed media, as shown in Table 1 and may also impose an osmotic stress at high solid concentration. These resulted in an adverse effect on biomass growth, at a 4:1 concentration ratio for both organisms. Both had an optimum biomass concentration at 3:1 concentration ratio. For *C. utilis* biomass there was little difference between the 3:1 concentrated and the non-concentrated medium (only a increase from 6,5 to 7,1 g/l biomass), while for *S. cerevisiae* biomass there was an increase from 3,3 g/l in the control medium (non-condensed) to 4,7 g/l in the 3:1 condensed medium (data not shown). However, sugar utilisation was not improved compared to the control (dephenolized medium without condensation), and maximum consumption of sugar could not exceed 15 g/l in all cases (data not shown). Thus, despite the addition of 5 g/l yeast extract there were limiting factors other than sugar concentration, which hindered biomass accumulation.

Effect of agitation rate

Three different processes were run at 150, 250 and 350 rpm to investigate the impact of agitation and mixing on biomass growth. For *S. cerevisiae* optimal stirring was at 350 rpm (aproximately 1 g/l increase in biomass compared to 250 rpm, and 1,8g/l increase compared to 150 rpm), while *C. utilis* biomass was

highest at 250 rpm with only a 0,5 g/l difference from the process at 150 rpm (data not shown). At 350 rpm C. utilis biomass dropped by 2 g/l, indicating an adverse effect of high stirring rate, possibly due to mechanical damage of cells (shear stresss), or oxygen-induced streess.

Addition of whey protein concentrate

In order to substitute the lacking nitrogen sources in OMW with a relatively cheap alternative originating from another organic by-product of the food industry, whey protein concentrate (WPC) from bovine milk (produced by ultrafiltration and condensation) was added to process medium at 6,25 g/l concentration. WPC contained approximately 80% protein (including low molecular weight proteinaceous compounds) and 15% lactose, thus the 6,25 g/l of WPC yielded 5 g/l protein in the process medium, equal to the amount of other nitrogen sources used previously, as well an extra 0,94 g/l of sugars (mainly lactose). The lactose content of the WPC is not fermentable by *S. cerevisiae* and *C. utilis*, however, residual glucose (and galactose) molecules present in WPC could be readily utilized. Its addition also increased the concentration of useful minerals, vitamins, etc in the process medium. S. cerevisiae was able to accumulate slightly higher biomass under these conditions (13,6 g/l), in comparison with *C. utilis* (12,15 g/l), possibly due to the process pH of 7, which was optimal for *S. cerevisiae*, and/or the activity and availability of galactose-hydrolysing enzymes in S. cerevisiae, which are absent in *C. utilis*, and can utilize the galactosidic moiety of formerly hydrolysed lactose molecules. For both organisms the use of WPC improved biomass growth significantly, compared to other nitrogen sources used previously. Sugar utilization was also slightly improved leading to a reduced 3,3 g/l of residual sugars at 79h in the *S. cerevisiae* process compared to previous processes.

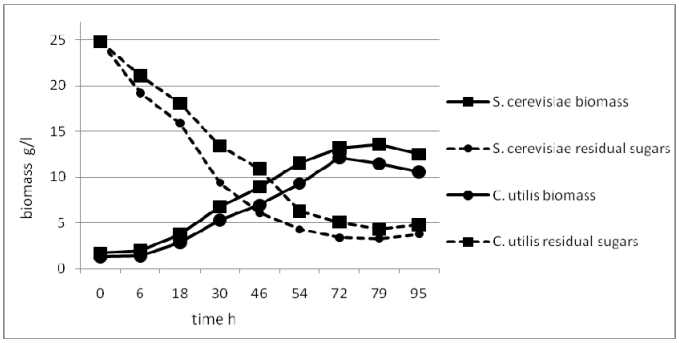


Figure 4. Time profiles of *S. cerevisiae* and *C. utilis* biomass and residual sugar concentration after addition of 15% deproteinized whey in dephenolized OMW. Process conditions: temperature 30°C, 250rpm, pH 7, 10% inoculum.

Protein content of the produced SCP

The crude biomass produced by edible yeasts can be used directly as animal feed, but for use in food, final protein concentration of the SCP is of great significance. Table 2 shows the mean values of protein concentration in different OMW based media. It exhibits that the addition of WPC increased the total protein content of the dry precipitate of SCP, whereas the condensation of the OMW lead to a decrease of the protein content of the SCP, possibly due to the accumulation of more solids in the medium, some of which maybe precipitated along with cell biomass, and thus reduce the percentage of pure protein.

Table 2. Protein concentration in SCP of *S. cerevisiae* and *C. utilis*, produced from three different OMW-based media (mean values of triplicate samples)

Type of substrate	Pure protein content in SCP (%)	
	S. cerevisiae	*C. utilis*
Dephenolized OMW	77,7	78,1
Dephenolized condensed OMW (3:1)	73,5	73,4
Dephenolized OMW with 6,25 g/l WPC	81,7	80,8

BOD-COD values

BOD and COD values decreased after dephenolization (~3 to 5-time reduction), and dropped further after fermentation and removal of biomass (data not shown).

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Effect of pulsed electric fields pretreatments on *S. cerevisiae* inactivation by high pressure carbon dioxide

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Abstract

The effect of coupling pulsed electric fields (PEF) as pretreatment to enhance the microbial inactivation of the high pressure carbon dioxide (HPCD) treatment was investigated.

McIlvaine buffer solutions inoculated with *S. cerevisiae* (CBS 1171) cells were pretreated with PEF at different field strength (E=6-12 kV/cm) and energy input (W_T=10-40 J/mL) and then processed with HPCD at pressures of 8.0, 11.0 and 14.0 MPa and contact times ranging from 3 to 30 min.

Results showed that treating microbial suspensions only with PEF (12 kV/cm, 20 J/ml, 25°C) the maximum inactivation level achieved was 0.35 Log-cycles. On the other hand, when yeast cells were treated only with HPCD, the inactivation level was almost independent on the processing conditions and never greater than 2 Log-cycles. The exposure of yeast cells to an external electric field prior to the application of HPCD treatment resulted in a marked increase of the inactivation level. Total inactivation was achieved when PEF (12 kV/cm, 20 J/mL, 25°C) was coupling as pretreatment to the HPCD processing (8.0 MPa, 25°C, 30 min).

A mathematical model based on the Weibull distribution adequately described the inactivation kinetics of *S. cerevisiae* cells treated with the combined process at different PEF treatment conditions and at fixed CO_2 pressure and temperature (8.0 MPa, 25°C).

Introduction

The current trend of the food industry is the individuation of milder preservation processes able to guarantee the food microbial safety without affecting its sensory attributes. To this extent, hurdle technologies have been developed as a new concept for the production of safe, nutrient, and tasty foods using less severe processing conditions. They are based on the combination of existing and/or novel preservation factors, applied in series or in parallel, providing additional or synergistic effects that no microorganism in the food should be able to overcome [1].

Pulsed Electric Fields (PEF) and High Pressure Carbon Dioxide (HPCD) technologies are two of the most promising non-thermal processing methods able to promote a cold pasteurization of liquid foods with a minimum impact on their nutritional and organoleptic properties [2, 3]. For both treatments the ability to inactivate vegetative microbial forms has been widely demonstrated [4, 5]. However in some cases it is necessary to apply intensive process conditions (high field strengths and energy inputs for PEF; high pressure, contact time and temperature for HPCD), to obtain substantial microbial inactivation ensuring food safety and stability [6, 7]. The combination of a PEF pretreatment with the HPCD process suggests the possibility to induce the electroporation of the cell membrane enhancing the CO_2 diffusion through the cell membrane during the following HPCD treatment in a way to obtain microbial inactivation at less severe process conditions.

Only two studies have been performed to investigate the advantages of coupling PEF with HPCD treatment for the inactivation of *S. cerevisiae* [8], *E. coli*, *S. aureus* bacteria and *Bacillus cereus* spores [9]. A synergistic effect between the PEF and the HPCD process has been reported for all the microbial

species investigated although further studies are necessary to better elucidate the inactivation mechanisms and the dependence of the inactivation kinetics on the process parameters of the combined treatment.

The present work investigates the effectiveness of a PEF–HPCD combined process on the microbial inactivation of *Saccharomyces cerevisiae* cells inoculated in a buffer solution. The effect of the PEF electric parameters (field strength and energy input) and HPCD processing variables (pressure and treatment time) on the inactivation kinetics of the yeast cells is evaluated and extensively discussed. A mathematical model based on the Weibull distribution is applied to fit the combined PEF and HPCD microbial kinetics.

Materials and Methods

S. cerevisiae (CBS 1171) cell culture was prepared by inoculating 1 mL of a preculture in 500 mL of sterile Malt Wickerham (MW) medium. The microbial suspension was then incubated at 25°C for 48 h in agitated conditions (130 rpm) until the early stationary phase was reached.
Before the experiments, microbial cells were recovered by centrifugation at 6000xg for 10 min at 4°C and re-suspended in a McIlvaine buffer solution (pH 7, k=2 mS/cm) to a final concentration of about $5 \cdot 10^7$ cfu/mL (colony forming unit/mL of sample).

Three different experiments of microbial inactivation were carried out: a single PEF treatment, a single HPCD treatment and a PEF pretreatment followed by a HPCD treatment.

PEF treatments with monopolar exponential decay electric pulses (pulse width 3.6 □s) of different field strengths (6, 9 and 12 kV/cm) and total specific energy input (10, 20 and 40 J/mL) were carried out in a laboratory scale continuous flow unit previously described by Pataro et al. [8]. Treatments were applied to the inoculated buffer solutions flowing at fixed flow rate (2 L/h) through the PEF chamber made of two parallel flat stainless steel electrodes with an area of 2.6 cm2 and kept at a distance of 0.25 cm with a Teflon insulator. Samples of the product were collected using sterile plastic test tubes, placed immediately in an ice-water bath before performing either the microbiological assay or the following HPCD process. In all the experiments the inlet temperature of the samples to the chamber was set at 25°C, while the measured outlet temperature was always lower than 33°C.

The HPCD experiments were carried out in a laboratory scale batch system [8]. The system consists of a vessel (100 ml in volume, maximum operative pressure 20.0 MPa) equipped with a four-bladed impeller that allows adjustable mixing speed and an electric heater connected to a temperature controller which rapidly brings the system to the desired temperature. During the treatments, 50 ml of either the untreated microbial suspension (single HPCD) or the PEF pretreated samples (flow rate 2 L/h, E=6-12 kV/cm, W_T=10- 40 J/mL) were poured into the vessel and afterwards liquid CO_2 (99.99% purity) was pumped into the reactor through an on-off valve by means of a volume displacement pump. The system was pressurized with the pump until the set pressure was reached. In all the tests a stirring speed of approximately 850 rpm was used to promote the dissolution of carbon dioxide in the liquid phase. Microbial inactivation experiments were carried out at fixed temperature (25°C) by exposing the microbial suspension at different pressure levels (8.0, 11.0 and 14.0 MPa) for different contact times (3, 5, 10, 20 and 30 minutes). At the end of each HPCD treatment, the system was depressurized and the samples were collected in sterile plastic test tubes, immersed in an ice-water bath before undergoing microbiological assay.

The number of surviving cells after each test was determined, after appropriate dilution of the treated and untreated samples in distillate water, by plates count method. The count of yeast colonies, grown on MW agar slants at 25°C for 62 h, was expressed as cfu/ml. Data were plotted as $\log_{10}$ of the survival fraction as a function of the HPCD processing variables.

A mathematical model (Eq.1) based on the Weibull distribution written in the power–law form [10] was used to fit the survival curves of yeast cells treated with the combined process at different PEF treatment conditions and at fixed CO_2 pressure (8.0 MPa) and plotted as $\log_{10}$ of the survival fraction (S) vs. contact time (t) between carbon dioxide and the sample.

$$\mathrm{Log(S)} = -\mathrm{b} \cdot \mathrm{t}^{\mathrm{n}} \quad (1)$$

where 'b' and 'n' were, respectively, the rate and the shape parameters obtained from the fitting of the experimental data using the least-squares criterion by the Solver function of the Excel 2007 package (Microsoft, Seattle, WA) and reported as a function of the processing variables.

Results and Discussion

The inactivation level of *S. cerevisiae* cells treated with PEF at different electrical field strength and total specific energy input is reported in Table 1. Results show that the PEF processing conditions applied are not able to induce an appreciable inactivation of the yeast cells. However, it is possible to detect a slight increase of the microbial inactivation when the field strength is increased from 6 to 12 kV/cm at a fixed energy input (20 J/mL), and when the energy input is increased from 10 to 40 J/mL at a fixed field strength (9 kV/cm), according to the theory of electroporation [11].

Table 1. Inactivation of *S. cerevisiae* cells by single PEF treatment

Log(N/N$_o$)	**E (kV/cm)**	**W$_T$ (J/mL)**
-0.084	9	10
-0.14	6	20
-0.16	9	20
-0.35	12	20
-0.26	9	40

Figure 1 reports the inactivation curves of *S. cerevisiae* cells achieved during the single HPCD treatment (0 kV/cm, 0 J/mL) and after PEF pretreatment (W_T=10-40 J/ml, E=6-12 kV/cm) followed by a HPCD process (8.0 MPa, t=0-30 min).

The HPCD treated cells show a non–linear inactivation kinetic. The inactivation rate is fast after few minutes of treatment and tends to level off to a relatively constant value (about 2.6 Log-cycles) with increasing the contact time, presenting the so called ''tailing behavior''. A possible explanation of this behaviour can arise from the existence heterogeneous treatment conditions due, for example, to a distribution of sensitivities to the HPCD treatment within the yeast population, or a poor diffusivity of liquid CO_2 under subcritical conditions.

Remarkable differences of the microbial inactivation level achieved are evident when comparing the results of the single HPCD and the combined PEF-HPCD treatments. The results reported in Figure 1 show that, yeast cells viability decreases during combined PEF-HPCD treatments with the increase of the field strength at a fixed energy input (Figure 1a) as well as with the increase of the total specific energy at a fixed field strength applied (Figure 1b). For a fixed treatment time of 10 min, the single HPCD process determines an inactivation of 2.49 Log-cycles. After the application of an electric field strength of 6 and 12 kV/cm at 20 J/mL, the inactivation level detected after the HPCD treatment is 3.91 and 6.98 Log-cycles, respectively. Similarly, an increase of the energy input from 10 to 40 J/mL during a PEF treatment at 9 kV/cm, yields an increase of the inactivation from 3.19 to 5.92 Log-cycles after the application of the HPCD process. This confirms the synergistic effect between PEF and HPCD processes. The complete inactivation of the *S. cerevisiae* cells is observed when the PEF pretreatment is carried out with an electrical field strength of 12 kV/cm and a total specific energy input of 20 J/mL and the HPCD process is operated at a pressure of 8.0 MPa and treatment time of 30 min.

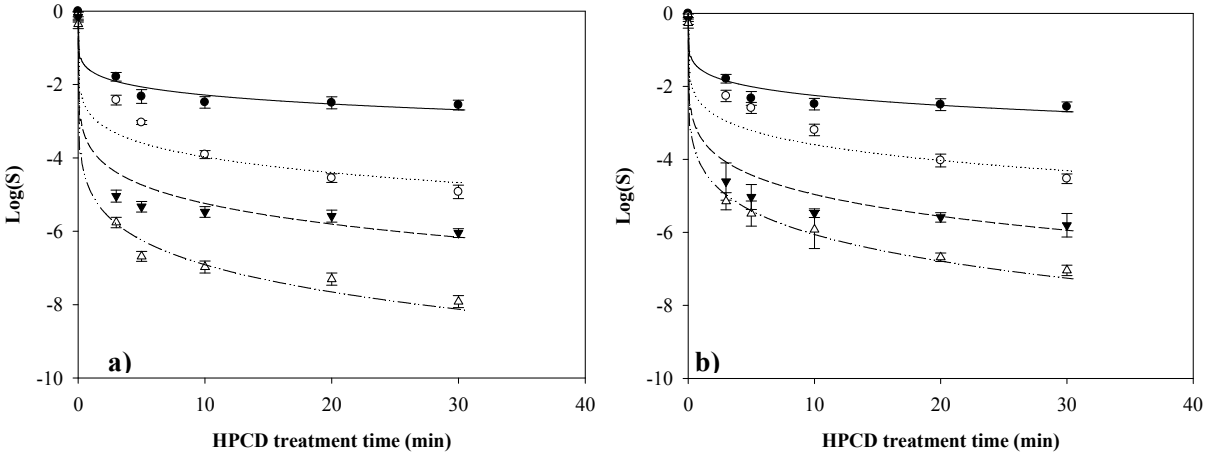

Figure 1. Inactivation kinetics of *S. cerevisiae* cells treated by combined PEF and HPCD (8.0 MPa and 25°C) process as a function of HPCD treatment time and at different PEF treatment conditions: a) W_T=20 J/ml, field strength of 0 kV/cm (●), 6 kV/cm (○), 9 kV/cm(▼), and 12 kV/cm (□); b) E=9 kV/cm; specific energy input of 0 J/ml (●), 10 J/ml (○), 20 J/ml (▼), and 40 J/ml (□). Symbols are experimental data, smoothed lines are the fit of the Peleg equation.

Results also highlight that, as already detected for single HPCD treatment, whatever is the PEF treatment intensity (E and W_T), yeast cells gradually become more CO_2 pressure-resistant with increasing the contact time.

The survival curves plotted in Fig. 1 were also fitted with Eq. (1). The estimated parameters b and n as well as the correlation coefficient (R^2) of each fitting are shown in Table 2. It is worth noting that the model is effective in predicting the yeast inactivation of combined PEF-HPCD processes within the PEF and HPCD treatment conditions investigated.

Table 2. "b" and "n" values from the fitting of the Eq. (1) to the survival curves of *S. cerevisiae* treated by PEF at different E and W_T.

W_T=20 J/mL				**E=9 kV/cm**			
E (kV/cm)	**b**	**n***	**R^2**	**W_T (J/mL)**	**b**	**n****	**R^2**
0	1.778	0.117	0.988	0	1.778	0.117	0.988
6	1.900	0.288	0.996	10	1.588	0.309	1.000
9	4.704	0.067	0.999	20	4.284	0.092	0.999
12	5.287	0.116	0.997	40	4.378	0.139	1.000

*mean value= 0.147; **mean value= 0.164

The 'b' parameter strongly depends on both the electrical field strength and the total specific energy input. On the contrary, the 'n' parameter is a weak function of both the electrical variables investigated. Therefore, the survival curves have been refitted with setting the n parameters equal to the mean values calculated for each PEF treatment investigated (Table 2). This makes possible to reduce the number of parameters of the model based on the Weibull distribution.

In the range of the investigated electrical conditions, the following relationship were observed between the re-estimated b values with both the field strength (Eq. 2) and the specific energy input (Eq. 3):

$$b = 1.629 \cdot e^{(0.0922E)} \qquad (R^2=0.995) \qquad (2)$$

$$Ln(b) = -0.00074 \cdot W_T^2 + 0.0544 \cdot W_T + 0.4317 \qquad (R^2=0.985) \qquad (3)$$

Eq. 2 and Eq. 3 were introduced in Eq. 1 in order to obtain the final model:

$$Log(S) = -\left(1.6292 \cdot e^{0.0922E)}\right) \cdot t^{0.147} \qquad (4)$$

$$Log(S) = -\left(e^{(-0.00074 W_T^2 + 0.0544 \cdot W_T + 0.4317)}\right) \cdot t^{0.164} \quad (5)$$

As it is possible to see in Figure 1 both the models based on the Weibull distribution fits well the inactivation and the shape of the survival curves of yeast cells.

Figure 2 reports the inactivation data of yeast cells treated in a combined process, with the PEF pretreatment stage carried out at fixed total specific energy input (20 J/mL) and different field strengths (6, 9 and 12 kV/cm) and the HPCD stage carried out at a fixed treatment time of 3 min and different pressure levels of CO_2 from 8.0 to 14.0 MPa. For the sake of comparison also the inactivation curve of yeast cells treated in the single HPCD process is reported.

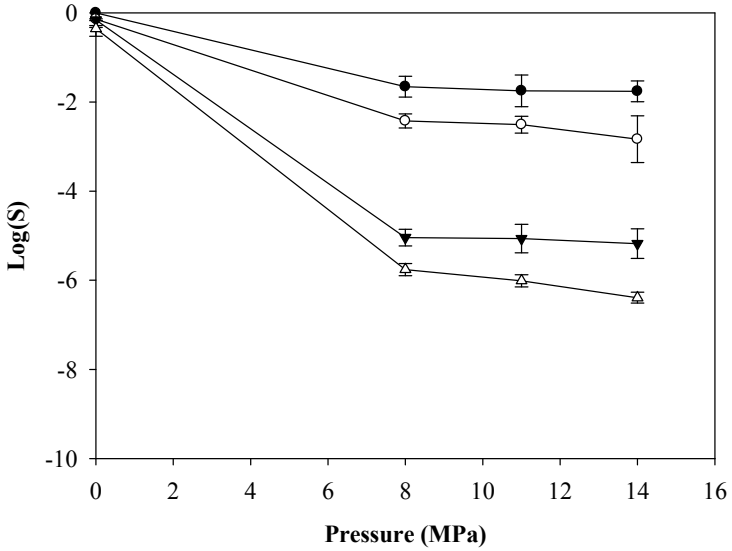

Figure 2. Inactivation of *S. cerevisiae* cells treated by combined PEF-HPCD process as a function of the CO_2 pressure. HPCD treatment at 25°C for t=3 min; PEF pretreatment at W_T=20 J/mL, and different electric field strengths: (●), 0 kV/cm; (○), 6 kV/cm; (▼), 9 kV/cm; (□), 12 kV/cm.

Results clearly show that in the single HPCD treatment the effect of the pressure of CO_2 is rather low in the range investigated. However, as the electric field strength applied to the microbial suspension increases, a significant enhancement of the inactivation rate following the HPCD process is detected. However, also in this case increasing the pressure the inactivation level does not increase whatever is the field strength applied. Changing the pressure from 8.0 to 14.0 MPa, CO_2 solubility in the liquid matrix increases but this concentration is probably not high enough to induce a detectable effect on the inactivation rate even after the PEF pretreatment.

The results obtained so far clearly show the existence of a synergistic action of PEF and HPCD process in microbial inactivation regardless of both the contact time between CO_2 and the sample and the CO_2 pressure applied in the investigated range. This can be attributed to the inactivation mechanism acting during the PEF and HPCD processes. It has been shown [9] that the HPCD process essentially involves the diffusion of CO_2 into the cells and that the diffusion mechanism is limited by the solubility of CO_2 in the suspending medium as well as by the mass transport resistance through the cell membrane [12]. The higher inactivation obtained utilizing the combined PEF-HPCD treatment is attributed to the permeabilization of the yeast cell membrane as a consequence of the application of the external electric field. According to the electroporation theory [11], applying more severe PEF treatment conditions (higher field strength and energy input), an increase of the degree of cell membrane permeabilization occurs, facilitating the diffusion of pressurized CO_2 into the cells.

Conclusions

The results demonstrate the existence of a clear synergistic effect combining PEF and HPCD treatments on the inactivation of *S. cerevisiae* cells. The combined process is able to ensure the total microbial inactivation at room temperature and at lower processing conditions with respect to the single PEF and HPCD treatments. Further experiments need to be carried out on real food matrices to evaluate the effects of the combined treatment not only on the microbial flora, but also on the physical and nutritional attributes of the products.

The Peleg model is able to fit with good agreement the inactivation kinetics of the cells treated with the combined process. Further experiments and modelling efforts needs to be done to take into account the dependence of the kinetic model's parameters on other processing variables such as CO_2 solubility and processing temperature.

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Effect of UV-C radiation on surface decontamination and the quality of black olive

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In this study, effect of Ultraviolet-C (UV-C) radiation on the surface decontamination and the quality properties of black *Gemlik* olive were examined. Black olives from *Gemlik* variety were purchased from an olive processing plant in İzmir, Turkey. UV light was produced using a laboratory scale UV apparatus. Aerobic plate count for bacteria and yeast and mould count were done to determine the surface disinfection efficiency. Hunter L*, a*, b* measurements, pH, titratable acidity and sensory evaluation were also done to determine other quality properties of olives. All analyses were carried out before and after treatments in order to determine the effects of UV irradiation on *Gemlik* black olive. All treatments were replicated three times and the results were statistically evaluated using the analysis of variance followed by Duncan's method with a significance level of $P< 0.05$.

The distance between the product and UV lamp and irradiation time were selected as parameters for UV irradiation. Three different distance (10, 15, 20 cm) and five different UV irradiation time (5, 10, 15, 20, 30 min) were applied.

According to the microbiological results, it is determined that as the UV irradiation time further increased and distance from the UV lamp decreased, inactivation effect of UV treatment increased. Exposure times of 10 and 15 min showed the same inactivation effect ($P\geq 0.05$) on the reduction of total aerobic bacteria. There was also no significant difference ($P\geq 0.05$) between 20 and 30 min. Considering further increasing of UV inactivation effect depending upon increasing exposure time, 20 and 30 min UV irradiation are more efficient ($P <0.05$) than others.

When examined distance between sample and UV lamps, although there was no significant difference ($P\geq 0.05$) between distances of 10 and 15 cm on the reduction of total aerobic bacteria, it was found that 10 cm more efficient than 20 cm ($P <0.05$), there were no significant difference ($P\geq 0.05$) between 15 and 20 cm.

The statistical analysis showed that there were no significant difference between exposure times of 20 and 30 min on reducing yeast and mould count but these exposure times (20 min and 30 min) were found more effective than others. 10 cm was found the most effective distance for reducing yeast and mould count on olives ($P < 0.05$).

In conclusion, among the used experimental conditions, the most effective conditions were determined as 20 min at 10 cm UV treatment. It was assigned that UV treatment did not affect on pH, titratable acidity and sensorial quality ($P \geq 0.05$). There were also no significant difference ($P \geq 0.05$) in Hunter L* and a* value. However, for irradiation times after 5 min there was significant difference ($P<0.05$) in b* value.

Isolation of microorganism strains for olive mill waste water treatment

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High phenolic compounds content process water from olive oil manufacturing (OMW -olive mill wastewater) represents one of the most important environmental problems in the Mediterranean region. Technologically and economically suitable solutions for the disposal of OMW have not been available until now.

This study aimed to isolate locally strains of anaerobic microorganisms can degrade the phenolic compounds present in the (OMW). The anaerobic microorganisms were isolated from (OMW) and from soil saturated with (OMW). The isolated microorganisms were assayed for its ability to degrade the phenolic compounds by incubation in cultures, each one contain one of the main tow phenolic compounds (Caffeic Acid and Protocatechuic Acid), commonly found in (OMW) as a lonely carbon recourse. Two different pH values were applied on the cultures 5.5 (pH of OMWW) and 7.0. The incubation was carried out at two different temperatures, 25°C and 35°C. The results indicated that the microorganisms were able to reduce the phenol concentration from 300 mg/l to low limits during 24 hrs of incubation. Four strains of higher ability to degrade the phenolic compounds were selected to assays its ability to degrade eight phynolic componds (Caffeic Acid, Protocatechuic Acid, Feruulic Acid, Vanilic Acid, Cinnamic Acid, Syringic Acid, Syring Aldehyde, Gallic Acid) in liquid medium with the phenolic compounds as single substrates.

The residual phenolic compounds in the culture were determined after 1, 3 and 5 days of incubation. The results show that in 5 days of incubation the isolated microorganisms were able to degrade 60-93% of phenolic compounds depending up on microorganisms strains and phenolic compounds. Classification of strains demonstrate that they are contributing to anaerobic clostridium

The results obtained indicate to:

Using specific locally strains of microorganisms is effective for degradation of phenolic compounds existing in OMW. Which lead to reduce the phenolic compounds contains to low levels then this treated OMW by microorganisms can be used in irrigations.

Microencapsulation of lemon peel essential oil in gum Arabic and maltodextrin mixture

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Microencapsulation is an important process in the food industry for improving chemical stability of key compound and formulating free flowing food powders. Capsulation materials protect the key components during processing, storage and consumption. Citrus oils are widely used in flavor, food, cosmetic, pharmaceutical and chemical industries. Lemon (*Citrus lemon* L.) peel essential oil is one of the flavor enhancing additives widely used in many food formulation. However, it needs to be capsulated with a convenient mixture of carrier materials for high value products. There are lots of carrier materials for microencapsulation processes, such as; starch derivatives, gums and proteins. Among these, gum arabic and maltodextrins mixture is the most common combination for encapsulating of essential oils.

In this research, lemon peel essential oil obtained by steam distillation was emulsified with six different ratios of arabic gum and maltodextrin combinations and processed into microcapsules by spray drying at preliminary tested condition. The obtained products were tested in physical (moisture content, water activity, bulk density, solubility, turbidity, product yield and encapsulation efficiency) and chemical (retention and composition of the essential oils) quality parameters.

Product yield, encapsulation efficiency, moisture content, water activity, bulk density and turbidity of the lemon peel essential oil were determined between 46-56 %, 72-89 %, 2.58-4.15%, 0.153-0.187, 382-404 kg/m^3 and 13.95-23.15 NTU, respectively. Results showed that the higher amount of maltodextrin in the emulsion leaded the higher in the product yield but, the lower in encapsulation efficiency. However, in all case, essential oil compositions were almost stable.

Breakup and coalescence of triglyceride droplets in plasticized starch matrices in simple shear flow

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Incorporation of functional lipophilic components into extruded starch based products has gained wide popularity, not only by food industry, but also by chemical, pharmaceutical, and medical industries. In many cases, regardless of type of lipophilic components to be incorporated, it is favourable to disperse the lipophilic components into small droplets of a lipid based delivery system to improve their stability, bioavailability and palatability. For this, either lipid droplets of target size have to be mixed into the starch based matrix in the extruder or the extrusion process itself can be used to disperse the lipid phase by breaking-up the droplets emerging from the mixing process. The latter process is more interesting from the cost point of view as it integrates several process steps to one unit, but requires a high degree of understanding of the mechanisms behind,

In laminar flow, as mainly found in extruders, shear and elongational stresses are responsible for droplet deformation and break-up. Resulting droplet sizes in addition depend on the viscosity ratio between dispersed and continuous phase. Since the starch is composed of long and partly branched molecules (i.e. amylose and amylopectin), it possesses non-Newtonian behaviour. Measurement of the viscosity and understanding the dispersion mechanisms under elongational and shear stresses inside the extruder is thus challenging. This knowledge, however, is the key to control resulting droplet sizes, and thus bioavailability and stability of lipophilic bioactive ingredients encapsulated within the oil droplets and the starch matrix.

Flow profiles within a co-rotating twin screw extruder, as used in this study, are very complex. In order to differentiate the influence of simple shear and elongation, experiments under controlled stresses were performed using an innovative shear cell developed by the Wageningen University and Research Centre (The Netherlands). With this device, plasticized pre-extruded starch was subjected to simple shear flow and low viscous oil (i.e. middle chain triglycerides) was added and dispersed. Thus, the influence of defined shear gradients on break-up and coalescence of oil droplets in plasticized starch matrices and resulting morphology of the product was investigated. Droplet size distributions were determined using confocal laser scanning microscopy and digital image processing. Cone/cone geometry of the shear cell exerts constant shear rates all over the flow domain; thus, allow measuring the viscosity curves of plasticized starch matrices in the same experiment. For the analysis of the droplet breakup, critical Capillary number was used and discussed in terms of non-Newtonian continuous phases.

Alpha-lactalbumin protein nanotubes

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Abstract

Protein and peptide based nanostructures promise novel technological opportunities in design, production and control. Recently, milk-based alpha-lactalbumin nanotubes (α-LaNTs) have been reported with potential applications. This study presents development and characterization of α-LaNTs due to self-assembly. Partial enzymatic hydrolysis and nanotube development was performed by incubating α-La and protease enzyme in the presence of calcium ions. Nanotubes developed were examined by microscopic techniques such as AFM and TEM. Peptides obtained during hydrolysis were identified by RP-HPLC and MALDI-TOF-MS. Dimensions of α-LaNTs obtained can be expressed as varying about 150 nm -1 μm in length and 20-40 nm in diameter. Retention time for α-La was about 55 min, and nearly all protein was hydrolyzed in 15 min, during incubation. Various sized peptides were obtained after enzymatic hydrolysis. Gelation properties were analyzed by reometer. Calcium-triggered α-LaNTs led to gel formation nearly after 30 min. Nanotube formation kinetics are underway. Also, encapsulation ability of formed nanotubes will be further investigated for some food applications.

Key words: α-lactalbumin, protein nanotubes, self-assembly

Introduction

Proteins and peptides extracted from various food sources are important matrices providing novel applications. Recently, food proteins have been investigated attractively for the ability of construction of nanoscale structures. α-Lactalbumin (α-La) is one of the major protein in whey. Alpha-Lactalbumin nanotubes (α-LaNTs) are such structures obtained by self-assembly of molecules after partial enzymatic hydrolysis [1]. They are also reported having potential industrial applications such as viscosifying, gelation and encapsulation purposes. Self-assembly can be identified by spontaneous diffusion and specific association of molecules through non-covalent interactions [2]. It is a 'bottom up' approach for nano structure production. Enzymatic hydrolysis leads to breakdown of proteins through smaller protein fragments and peptides depending on the degree of hydrolysis. Then, naturally occurring self-assembly gives rise to fabrication of novel structures from these fragments. These types of nanostructures can be analyzed by microscopic techniques to identify their morphologies and dimensions. Especially, Transmission Electron Microscope (TEM) and Atomic Force Microscope (AFM) were mostly used for such identifications. The characteristics of the peptide fragments formed after enzymatic hydrolysis have great significance to identify the mechanism of development of the peptide-based nanostructures. High Performance Liquid Chromatograph (HPLC) and Mass spectrometry (MS) techniques are available to identify these peptides.

This study presents development of α-LaNTs due to self-assembly; analysis of nanotubes by AFM and TEM, and partial profiling of peptides formed during hydrolysis and nanotube formation by HPLC and MALDI-TOF MS. Also, gelation property of these nanotubes was investigated by rheometer.

Materials and methods

Partial enzymatic hydrolysis and nanotube development was performed by incubating α-la (Sigma L6010) and BLP (Novozymes) in the presence of calcium ions [1]. Nanotubes developed were examined by AFM and TEM. Peptides obtained during hydrolysis were investigated by RP-HPLC [4] and MALDI-TOF MS. The column used was Agilent C18, 4.6 X 25 cm. The matrices used were 2,5 di-hydro benzoic acid (DHB) and α-cyano 4-hydroxycinnamic acid (CHCA).

Results and discussion

Alpha-lactalbumin nanotubes were developed by self association of peptide fragments after partial hydrolysis. Dimensions of α-LaNTs obtained can be expressed as varying 150 nm-1µm in length and 20-40 nm in diameter, nearly. Figure 1a and 1b represents an AFM and TEM image of α-LaNTs, respectively.

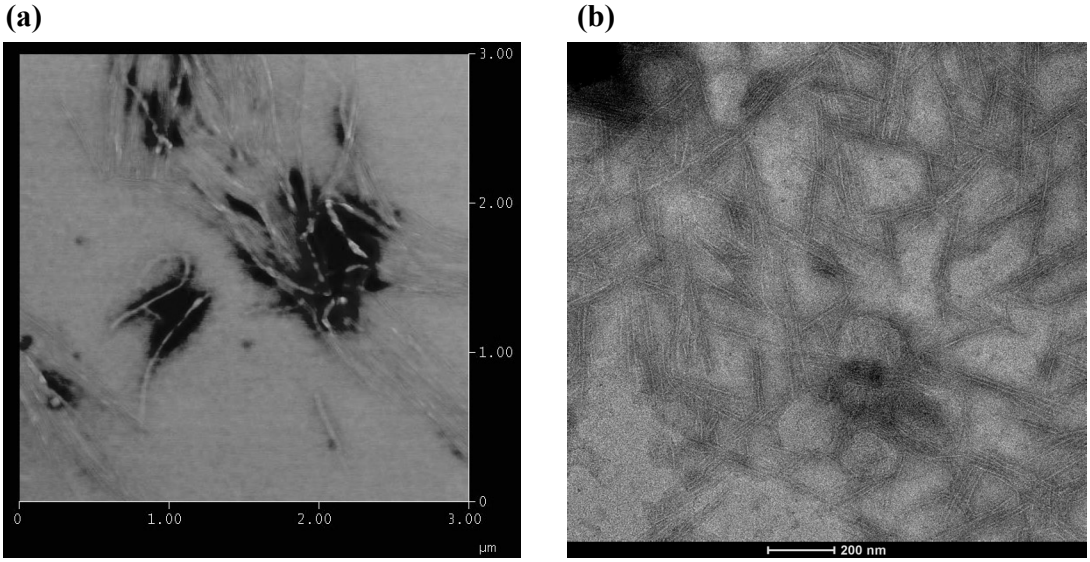

Figure1. AFM **(a)** and TEM **(b)** images of α-La nanotubes (α-LaNTs)

The nanotubes were in linear fashion mostly, but some of them were also slightly curved (figure 1a). They seemed in a bundle form, and very few individual was observed in AFM images whereas they seemed more individually in TEM images. This may be related with sample preparation. Graveland-Bikker et.al. analyzed self-assembled α-LaNTs by scanning force microscope (SFM). They reported that these nanotubes with 1µm length and 20nm width [6]. The AFM images obtained in this study is comparable to SFM image presented by them. Also, TEM images obtained in this study are very similar to that of repoted in their study.

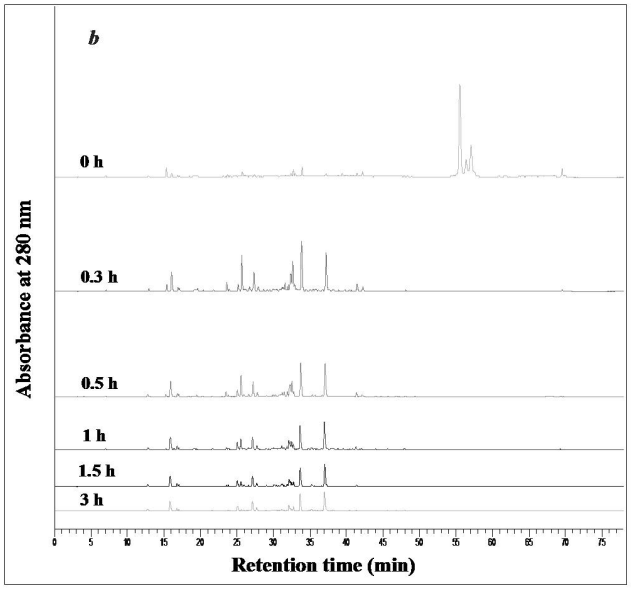

Figure 2. RP-HPLC chromatogram of peptides during hydrolysis

Peptides formed during enzymatic hydrolysis were investigated by RP-HPLC and MALDI-TOF MS. Chromatograms of the hydrolysates at various times of incubation were given in figure 2. At the beginning of incubation (0 h), non-hydrolyzed α-la peak was seen clearly. Retention time for α-La was about 55 min, and nearly all protein was hydrolyzed after 20 min, during incubation. Various sized peptides were obtained during enzymatic hydrolysis.

Mass spectra of hydrolysis products in the first 15 minutes were given in figure 3. The molar mass of α-la is about 14.2 kDa. Mass spectra were taken from 0h, 0.25h 0.5h, 1h and 1.5h samplings firstly. However, the α-la peak disappeared in the samples for 15 minute or longer incubation times (data is not given). Therefore, spectra in the first 15 minute were detected by one minute intervals.

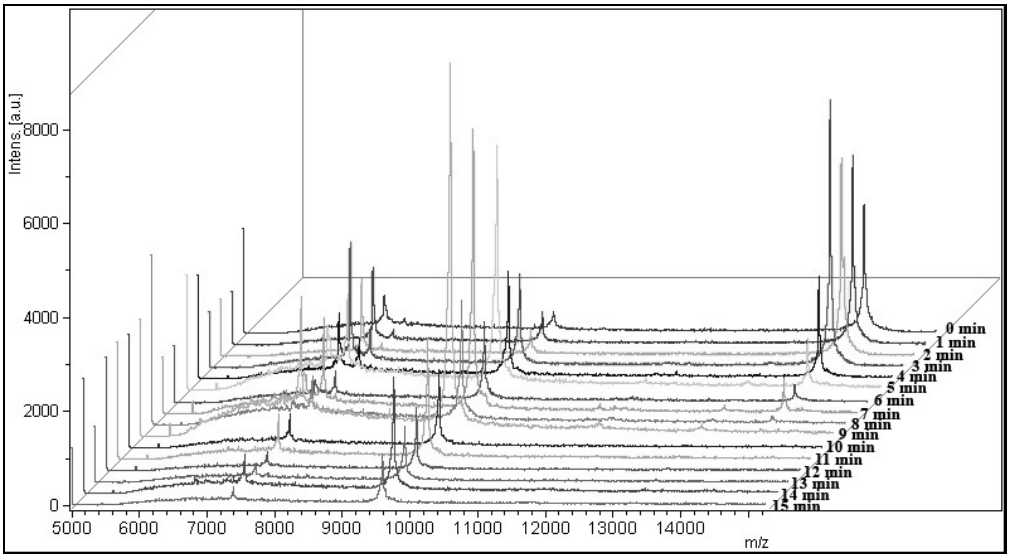

Figure 3. Mass spectra of peptides during hydrolysis

Figure 3 indicates that, no α-la peak was observed in the spectrum of 9 minute-sampling. Some researchers investigated the peptides obtained by enzymatic hydrolysis of α-la by liquid chromatography mass spectrometry (LC-MS) [1], [4]. They reported both chromatograms of peptides detected at certain time intervals during incubation by LC and their masses by MS. In this study, hydrolysis of α-la and formation of new fragments depending on time were presented firstly. It is obvious that α-la is hydrolyzed quite fast by BLP at given conditions. Whether the nanotube formation is similar speed should be investigated, also. Further analysis on peptide characterization, including LC-MS by samplings during hydrolysis, is underway.

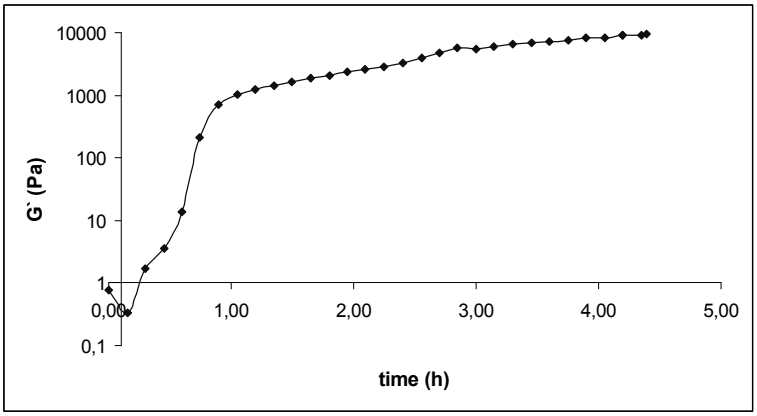

Figure 4. Storage modulus of α-La incubated with BLP and calcium ions

Figure 4 indicates storage modulus, G` plotted as a function of time. Gelation was defined at the point that G` was greater than 1 Pa. According to this, gelation occurred nearly after 30 minute during incubation. Actually, calcium ions trigger nanotube formation. When calcium was not added to incubation mix, no nanotubes were formed, and also gelation was obtained after nearly 10 hours (data not shown).

Conclusion

In this study, the production of α-LaNTs due to self-assembly after enzymatic hydrolysis and partially, peptide analysis were given. AFM and TEM images indicated α-LaNTs with nearly 150 nm-1 μm length and 20-40 nm width. Mostly, they appeared in a linear fashion and bundled form. Peptide profiles during hydrolysis assigned non-hydrolyzed α-la peak at the beginning of reaction disappeared readily and various sized peaks appeared. In addition, nanotube formation fastened gelation. More study will provide better comprehension on α-La hydrolysis and formation of nanotubes. Kinetic studies to understand nanotube development are planning for the next. Besides self-assembly, leading to random development, more controlled other techniques for nanotube growth are also ongoing.

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Microencapsulation properties of angom gum for the emulsification of D-limonene droplets compared with arabic gum

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Development of alternative and inexpensive polymers that may be considered natural, like Arabic gum, and that could encapsulate e.g. flavours with the same efficiency than Arabic gum are an area of research of increasing interest. The emulsification properties of Angom gum (An) for use as a flavour encapsulant in spray drying encapsulation was investigated in this study and the results were compared with Arabic gum stabilized emulsions (Ar).

One day before emulsification, wall material powders were dissolved in distilled water and kept overnight to warrant a full saturation of the polymer molecules. Total concentration of dissolved solid was 40% (w/w) composed of (35-39) wt% maltodextrin and (1-5) wt% emulsifying ingredients (An or Ar). Pre-emulsions (oil-in-water) were produced by an Ultra-Turrax at 20,000 rpm and the core material (d-limonene) in percentage of 5 and 10 was progressively added to the continuous phase during pre-emulsion preparation. These coarse emulsions were then further emulsified using a high Pressure homogenization at 250 bars. Emulsion stability was determined by storage time, particle size by using master sizer and morphology by light microscopy.

At the same concentration, Angom-emulsified d-limonene droplets were most stable and Arabic-emulsified d-limonene droplets were least stable during 3, 15 and 30 days of storage at room temperature. Statistical analysis of master sizer results revealed that there is a significant difference between flavour level, gum type and particle size at α=0.05. The results showed that increasing the gum level causes an increase in droplet size. Furthermore, master sizer results revealed that Angom-emulsified droplets at 2% gum and 5% flavour level had the lowest D_{32}, D_{43} and the highest specific surface area by high-pressure homogenizer. So, these figures are the optimum level of An in emulsification of 5% D-limonene, while Ar is 5%.

Based on this study, Angom gum must be considered as a novel suitable wall material for microencapsulation of food ingredients by spray-drying. Results in this work showed that the ability of Angom gum to encapsulate d-limonene were comparable with Arabic gum so that Angom-emulsified droplets at 2% gum was the optimum level of Angom for 5% flavour level.

Development of a rapid analytical method for determination of total polyphenols in plant material used for meat production

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Abstract

Polyphenols are chemical compounds, belonging in the class of antioxidants, which have attracted extensive attention as natural bioactive materials. The availability of polyphenols in cheap natural sources such as tissues of several plants makes their utilization more attractive and favourable. The aim of the proposed research work was to develop a new technique for measurement of total polyphenolic compounds as Gallic acid and to recover these polyphenolic compounds from tissues of fobber plants such as *Petroselinum crispum***,** *Poa pratensis, Vicia sativa, Medicago sativa, Trifolium sp., Hordeum vulgare, Avena sativa* and *Triticum cereale*. There is a significat difference between various plant tissues and pasturage products in the percentage of total polyphenols contained in their tissues. From the eight different plants tested for polyphenolic compounds, *Petroselinum crispum* and *Poa pratensis* was found to have the highest concentrations while the lowest polyphenol concentration was found to be in *Triticum cereale* tissues. According to the developed UV method the peak of absorption presented at 765nm and the linear range was from 0-50 ppm. The validation tests of the developed rapid method proved that the repeatability was satisfactory so that this can be suggested for routine analysis of several plant tissues.

Introduction

The interest for the role of the natural antioxidant compounds in human and animal health has been increased the last few years. The interest of the researchers is focused in the creation of natural plant products which contains high concentrations in antioxidant substances, adding additional nutritious value of foods. Among the substances that have been found to appear antioxidant action are various phenolic and polyphenolic compounds such as simple phenols, flavonoids terpenoids etc found in several common plant tissues and products.

Antioxidants are compounds that can delay, inhibit, or prevent the oxidation of oxidizable matters by scavenging free radicals and diminish oxidative stress. Plants contain a wide variety of antioxidant phytochemicals or bioactive molecules, which can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress. The intake of natural antioxidants has been associated with reduced risk of cancer, cardiovascular disease, diabetes and diseases associated with ageing. Studies on dietary free radical scavenging molecules have attracted the attention to characterize phenolic compounds and other naturally occurring phytochemicals as antioxidants (Ani et al., 2006).

Moreover, plants encounter numerous pests and pathogens in the natural environment. An appropriate response to attack by such organisms can lead to tolerance or resistance mechanisms that enable the plant to survive. Thus, most plants produce a broad range of secondary metabolites that are toxic to pathogens and herbivores, either as part of their normal program of growth and development or in response to biotic stress. Among the metabolites in nature with the above characteristics are Polyphenols. It is agreed that phenolic compounds are widely distributed in plants used for defensive functions showing antimicrobial activities (Boudet 2006; Xia et al., 2010) controlling bacteria [Baydar et al., 2006; Taguri et al., 2004; Ani et al., 2006), fungi (Bruno and Sparapano 2007) and viruses (Chavez et al., 2006). Thus, there is currently an increasing interest in the isolation, examination and exploitation of agricultural wastes or inexpensive plant sources, rich in polyphenols such as tissues of *Olea europaea*, *Prunus amygdalus, Stevia rebaudiana* and in their wastes such as olive mills waste waters. Phenolics are a class of plant secondary metabolites that contain one or more hydroxyl derivatives of benzene rings.

Furthermore, according to Manach et al., (2005), polyphenols are common constituents of the human diet, present in most foods and beverages of plant origin. They are considered to contribute to the prevention of various degenerative diseases, including cardiovascular diseases. Two reasons in particular can be inferred. The first one is that the polyphenol family encompasses very diverse compounds with highly different bioavailabilities. Hence the results obtained for one polyphenol cannot be generalized to others. The second point is that polyphenols are now known to be largely metabolized in the body and native compounds most often tested in *in vitro* studies are virtually absent in the tissues.

The study of polyphenols has begun in 1950 and up to the beginning of next decade an important part of chemistry of the polyphenolic compounds had become acquaintance. Nevertheless, the available analytic techniques and the limited knowledge of these compounds did not allow a completed approach on to this subject. Hardly in 1976 was analyzed the quantity of polyphenols in the foods with chromatography of thin stack (Thin Layer Chromatography, T.L.C), while enough later, in 1992, were studied the total content in aglicones from five flavonoids and flavones in fruits and vegetables and tea. Since then they have been recognized thousands flavonoid compounds and continuously are isolated new. Since then high concentrations of catechins, flavonols and depsides were found to be restricted to the young vegetative and floral shoots, whereas leucoanthocyanins r flavylogens were characteristic of the more bulky axial tissues of the plant.

Polyphenols are enough widespread in the plant foods such as vegetables, cereals, legumes, dry fruits, fruits as well as in the drinks such as wine, beer, tea, cocoa etc. The quantity of total polyphenols presented varies even between cultures of the same type. It is known that the quantity of the total polyphenols in plant tissues is depending on genetic factors and by the environmental conditions. Other factors as the degree of maturation the variety, the treatment and the storage, influence the content of phenolic compounds derivatives of plants. The bitter flavor of foods and drinks depends from their content in polyphenols. In the legumes and the cereals the most common polyphenolic compounds are flavonoids, phenolic acid and tannines. Furthermore, in the legumes the higher amount of content in polyphenolic compounds appeared to be in the dark varieties such as red and black beans. The legumes contain also isoflavones while the vegetables contain mainly flavonoids. The roots and the tubers have low concentrations of flavonoids, with the exception of certain plants as the onions and the liquorice.

In the past, analytical methods used for search of total phenols in the animal diet have demonstrate many examples of inadequate use of them. Precautions should be taken at the export of phenols (McLeod 1974, Gartlan et al., 1980). Many phenolic unions affected by the sunlight, react with oxygen in the alkaline solution and with methanol in the temperature of room and the pH 6 (Haslam, 1966). The existence of phenols in the plants has been measured colorimetrically using the reaction agent folin Denis (Herdsman and Hillis, 1959). Furthermore, this reaction agent has been often used in order to measure the content in tannin in the harvests of fodder crops due to its simplicity in use (Burn, 1963).

The reaction agent of folin as it is modified from Folin and Ciocalteau (1927) gives a better estimate of total phenolic compounds (Singletone and Rossi, 1965). This reaction agent gives a better answer of colour with phenols and a smaller answer in the not-phenolic unions. (Price and Butler, 1977).

Materials and Methods

For the determination of total polyphenols extracted for the plant tissues were used the following materials:

Folin-Ciolteau: Phenol reagent 100 ml F-9252 Lot 52K3671
Gallic acid: $C_7H_6O_5H_2O$ 1-hydrate chemically pure 500g 2311K Lot 219862100
Sodium carbonate: Na_2CO_3 anhydrous (Reag. Ph. Eur) PA-ACS-ISO 1000g
Ethanol: absolute PA-ACS-ISO CH_3CH_2OH 2.5 L
Distilled water

The plants that they were used is this study, are mainly veterinary plants used for the diet of animals which are either cultivated or are found in the countryside. These plants participate also in the human diet. The samples that were taken were fresh plan tissues and no seeds at all. The plants used in this study were species of *Medicago sativa*, *Hordeum vulgare*, *Vicia sativa*, *Petroselinum crispum*, *Avena sativa*, *Poa pratensis*, *Trifollium* sp and *Triticum* spp.

The determination of total polyphenols becomes with the method known as Folin Ciocalteu. At this method is prepared solution Gallic acid and sodium carbonate.

Preparation of solution of Gallic acid: In volumetric bottle of 100ml they were dissolved: 0,5gr Gallic acid in 10ml of ethanol. Complete with distilled water up to final volume.

Preparation of solution of sodium carbonate: In 2 lt glass volumetric flask were dissolved: 200gr of arid carbonic sodium and 800ml of distilled water. Furthermore, the solution was placed on a thermal plate, and brought to boiling continuously stirred. Consequently the solution was cooled down and then certain crystal of sodium carbonate was added. After 24 hours the solution was filtered and distilled water was added in the volumetric flask of final volume of 1lit.

After this procedure it followed dilution of the initial solution in volumetric flasks of 50ml where they were placed 10ml from the solution of Gallic acid. Finally the 50ml flask was filled by distilled water. Then, in 5 volumetric flasks of 25ml they were placed 1ml 2ml 3ml 4ml 5ml from the diluted solution of Gallic acid respectively and distilled water was supplemented up to final volume. In 5 test pipes they were placed 1,6ml from each volumetric flask (1,2,3,4,5), 0,1ml Folin- ciolteau which was left for 8 min, 0,3gr of sodium carbomate. All samples were stirred and churned in the vortex. From the above solution 5 samples were made and theywere placed in dark environment with temperature 20ºC for 2 hours. It follows churn in the vortex. Afterwards, the UV absorption was measured at 765nm compared against a blank solution which corresponded in the volumetric flask that did not contain solution of Gallic acid.

Extraction of polyphenols from plant tissues: 1 g of sample was extracted for two hours with 20ml of solution of 80% of ethanol that contains 1% HCl under continuous stir at 200 rpm in room temperature. The mixture was centrifuged at 3000 rpm for the 20 minutes and the supernatant solution was transported in the test pipe Falcon (50ml). The solid residual in the Falcon tube was treated once more by centrifugation in a similar way. The two supernatant solutions were transferred into 50ml volumetric flask and alcoholic solution was added up the volumetric line.

Preparation of the sample: In volumetric flask of 25 ml 2,5 ml of the diluted ethanolic extract of the plant tissue were added. The solution supplemented with distilled water up to final volume. Afterwards, in test pipes of 5 ml, 1,6ml of the final alcoholic solution – prepared sample 0,1ml Folin-Ciocalteu reagent and within a period of 8min 0,3ml of solution of sodium carbonate was also added. The samples brought up well and were placed in the dark for one hour. Afterwards, the absorption was measured at 765nm compared against a blank solution which corresponded in the volumetric flask that did not contain solution of Gallic acid.

For the calculation of the concentration of total polyphenols in the plant tissue the following formula was used: ppm of total polyphenol in the plant tissue = 500 x concentration of measured solution.

Results and Discussion

From the eight different plants tested for polyphenolic compounds, *Petroselinum crispum* and *Poa pratensis* was found to have the highest concentrations while the lowest polyphenol concentration was found to be in *Triticum cereale* tissues. According to the developed UV method the peak of absorption presented at 765nm and the linear range was from 0-50 ppm. The validation tests of the developed rapid method proved that the repeatability was satisfactory so that this can be suggested for routine analysis of several plant tissues.

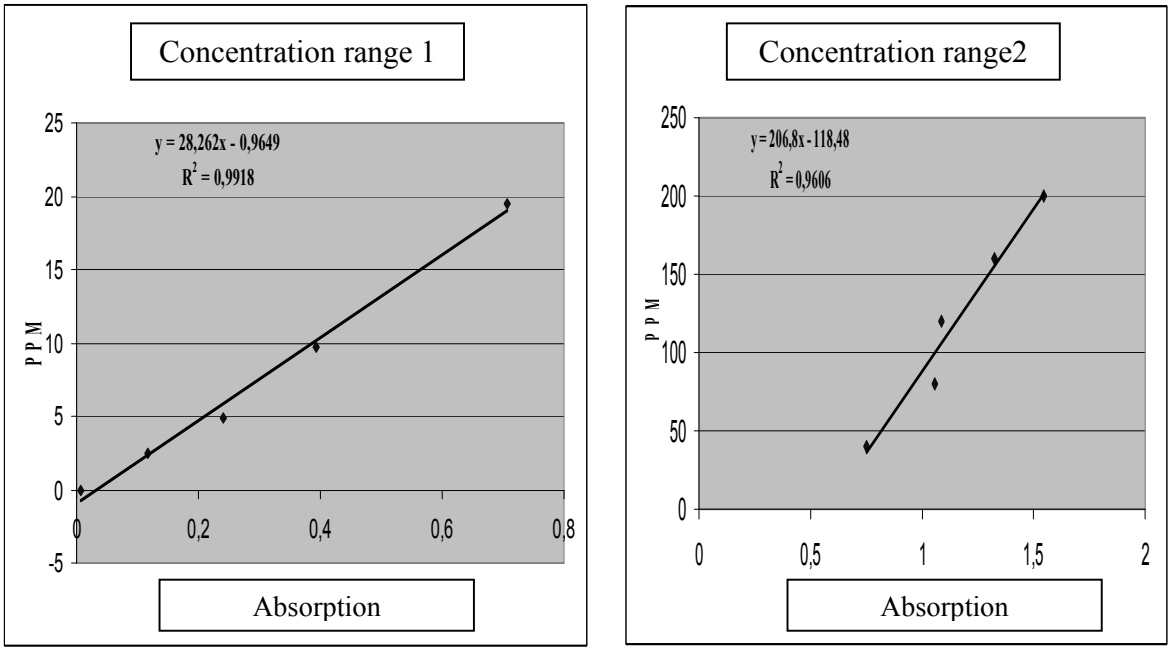

Figure 1. The calibration curves for two respective polyphenol concentration ranges

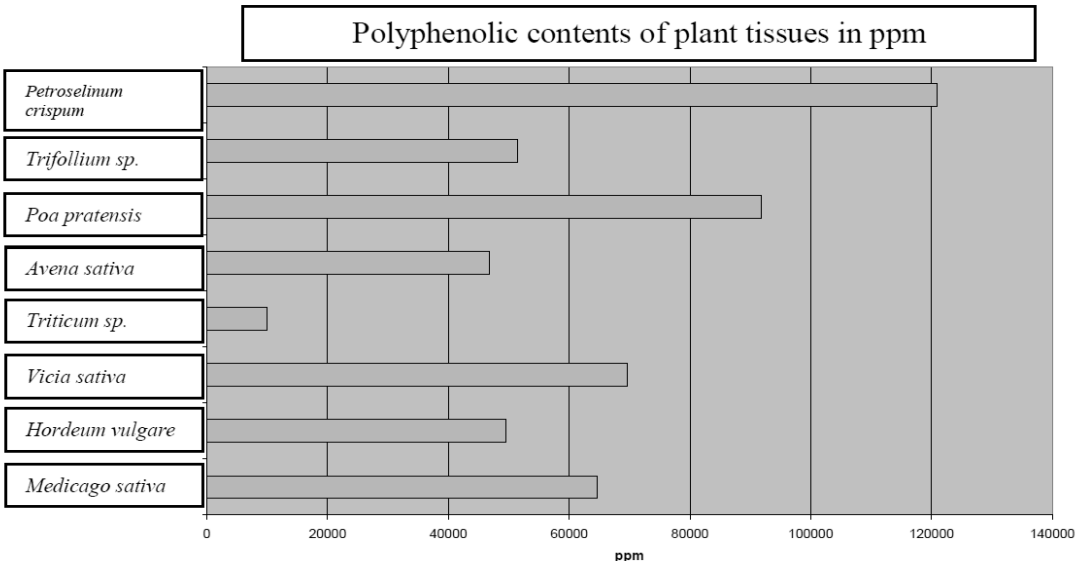

Figure 2. Polyphenol concentration measured in various plant tissues.

Conclusions

1. There is statistically important difference in the percentage of the polyphenolic compounds between various plant tissues.
2. The higher concentration of polyphenolic compounds was observed in the parsley and was 120880ppm on dry bases and the lower in wheat sample and it was 9900ppm on dry bases.
3. The region of linearity of curve of absorption was from 0 up to 50ppm nevertheless can be used with linear adaptation and other regions of curve. Ideally in future measurements the samples should diluted so as to they are in region 0-50 ppm
4. The highest absorption was observed at the 765nm and it is the wave length that should used for the method.

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Ultra performance liquid chromatography (UPLC™) analysis of biogenic amines in cheese

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Abstract

Biogenic amines are produced in cheese by enzymatic decarboxylation of amino acids. High levels of biogenic amines can result in food poisoning, and cases of histamine intoxication have occurred subsequent to the consumption of cheese. The objective of this study was to establish a *pre-column* derivatization method to determine biogenic amines in cheese. AccQ-Fluor derivatizing reagent (6-Aminoquinolyl-N-hydroxy-succinimidyl carbamate) was used to analyze primary and secondary biogenic amines by ultra performance liquid chromatography (UPLC™). A reliable method was established for the simultaneous separation of 20 biogenic amines in cheese within 9 minutes.

Commercial cheese samples from retail outlets in Vienna were analyzed for their biogenic amine content, that varied to a great extent, depending not only on the type of cheese, but also within a certain cheese variety. About 13.8% of samples had histamine, and 22.4% had tyramine content above 100 mg/kg. The highest concentration of histamine was found in Tiroler Almkäse (1160 mg/kg) and Vorarlberger Bergkäse (397 mg/kg), the highest amount of tyramine was found in Cantal, Olmützer Quargel and Tiroler Almkäse (about 470 mg/kg). Moreover, 8.6% of samples had a putrescine or cadaverine content higher than 100 mg/kg. The highest concentrations were detected in Olmützer Quargel (523 mg putrescine, 748 mg cadaverine per kg). Thus, the total concentration of biogenic amines in some samples was about 1940 mg/kg. In conclusion, the fast and reliable UPLC™ method to determine biogenic amines in cheese represents a valuable tool to ensure quality of dairy products.

Introduction

Proteolysis is the principal and most complex biochemical event occurring during maturation of most cheese varieties, being of high significance for the development of texture and flavour of the final product. In most cheese varieties, proteolytic changes occurring during cheese ripening directly contribute to flavour and perhaps to off-flavour (e.g., bitterness) of cheese due to the formation of peptides and free amino acids (FAA). Furthermore, proteolysis plays an important role in the liberation of substrates for secondary catabolic changes (e.g., deamination, decarboxylation, transamination). The concentration of FAA depends on the cheese type and has been used as an index of ripening age. The relative proportions of individual amino acids are thought to be important for the development of the characteristic flavour of a cheese variety. Biogenic amines are produced in cheese by enzymatic decarboxylation of free amino acids (Fox, 1993).

Biogenic amines are aliphatic, alicyclic and heterocyclic organic bases of low molecular weight. These substances are ubiquitous in biological materials and are not only biosynthesized in animal and plant cells, but are also produced via decarboxylation activities of bacterial enzymes. Biological systems (e.g., in fermented foods) are ideal substrates for amine production, as ongoing biological processes involve not only available free amino acids, but also the presence of decarboxylase-positive microorganisms and environmental conditions appropriate for the growth of microorganisms and the action of decarboxylase enzymes (Askar and Treptow, 1986).

Biogenic amines have various toxicological implications (e.g., histamine intoxication, hypertensive crises, and migraine headaches). However, biogenic amines are not considered a grave risk for humans if they are

present in low levels in foods and if their metabolism is not blocked or genetically altered. Small amounts of biogenic amines can usually be found in some foods, because they play a natural role in microbial, plant and animal metabolism. However, some amines such as tyramine, histamine and serotonin, can also have direct or indirect effects on the human vascular and nervous system. Other biogenic amines such as putrescine and cadaverine, although they do not pose a direct risk, can also have negative effects on health since their action stimulates the toxicological effect of tyramine and histamine. Thus, the consumption of large amounts of these biogenic amines can lead to undesirable effects as headache, nausea, hypo- or hypertension, cardiac palpitations, and anaphylactic shock syndrome. These effects can be especially adverse in patients treated with classical monoamine oxidase inhibitors drugs or in individuals with genetic or acquired diaminoxidase deficiency. Therefore, the analysis of biogenic amines is of interest for their toxicological risk, but also as an indicator of food quality (e.g., in estimating freshness or degree of spoilage of fish). As biogenic amines have been proposed as possible indicators of poor hygienic quality of raw materials used and/or poor manufacturing conditions, some countries have established legal limits or at least tolerable maximum contents for histamine in fish and fish products (100 mg/kg). In cheese, biogenic amines are produced by enzymatic decarboxylation of free amino acids. The principal amines in most cheeses are tyramine and histamine produced by decarboxylation of tyrosine and histidine, respectively. High levels of biogenic amines can result in food poisoning, and cases of histamine intoxication have occurred subsequent to the consumption of cheese (Askar and Treptow, 1986; Beutling, 1996).

Different methods have been used to determine biogenic amines. Since most amines show neither natural UV absorption nor fluorescence, most methods require that amines should be derivatized before detection, or indirect detection can be used. Different chemical regents have been used for the amine analysis, e.g., ninhydrin in amino acid analyzers as a post-column derivatization reagent, danysl-chloride with pre-column derivatization, o-phthalaldehyde, fluorescein isothiocyanate, phenyl isothiocyanate, 9-fluorenyl methyl chloroformate, benzoylchloride and fluorescamine. For the separation of biogenic amines, various chromatographic techniques such as thin-layer chromatography, gas-liquid chromatography and HPLC as well as capillary electrophoretic methods are used. The separation of underivatized amines by ion-pair chromatography is also a well-known technique, but it has the drawback that amines such as putrescine and cadaverine cannot be detected as they lack a suitable chromophore, whereas others like histamine must be detected at wavelengths that are not selective, so the risk of interfering matrix components cannot be neglected. In order to prevent matrix interferences and to enhance detection sensitivity when using HPLC, derivatization reactions with Dns-Cl and OPA are commonly used. However, the use of most of these reagents involves a number of drawbacks, as Dns-Cl is a non-specific reagent reacting also with phenols, alcohols and some sugars, and OPA reacts with primary amines only. To avoid the drawbacks of reagents such as Dns-Cl and OPA, AccQ-Fluor derivatizing reagent (6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate; AQC) has already been reported as being of use in the *pre-column* derivatization of primary and secondary amino acids and biogenic amines (Veciana-Nogues et al.,1995; Busto et al., 1996; Novella-Rodríguez et al., 2000, 2003).

Ultra performance liquid chromatography (UPLC™) is a new category of analytical separation science that retains the practicality and principles of HPLC, while increasing the overall interlaced attributes of speed, sensitivity and resolution. During the past 30 years, HPLC has proven to be the predominant technology used in laboratories worldwide. One of the primary drivers for the growth of this technique has been the evolution of the packing materials used to effect the separation (Swartz and Murphy, 2004). The underlying principles of this evolution are governed by the *van Deemter* equation (Figure 1). The *van Deemter* equation is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). Since particle size is one of the variables, a *van Deemter* curve can be used to investigate chromatographic performance. Figure 1 demonstrates that as the particle size decreases to less than 2.5 µm, there is not only a significant gain in efficiency, but the efficiency does not diminish at increased flow rates or linear velocities. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits, termed Ultra Performance Liquid Chromatography. Using UPLC, it is now possible to take full advantage of

chromatographic principles to run separations using shorter columns, and/or higher flow rates for increased speed, with superior resolution and sensitivity (Swartz and Murphy, 2005; Ferse et al., 2006).

The objective of the present study was to establish a reliable and rapid UPLC™ method using AccQ-Fluor precolumn derivatizing reagent (6-Aminoquinolyl-N-hydroxy-succinimidyl carbamate; AQC) for the simultaneous determination of twenty primary and secondary biogenic amines in cheese. Commercial cheese samples from Austrian retail outlets were to be analysed regarding their biogenic amine content.

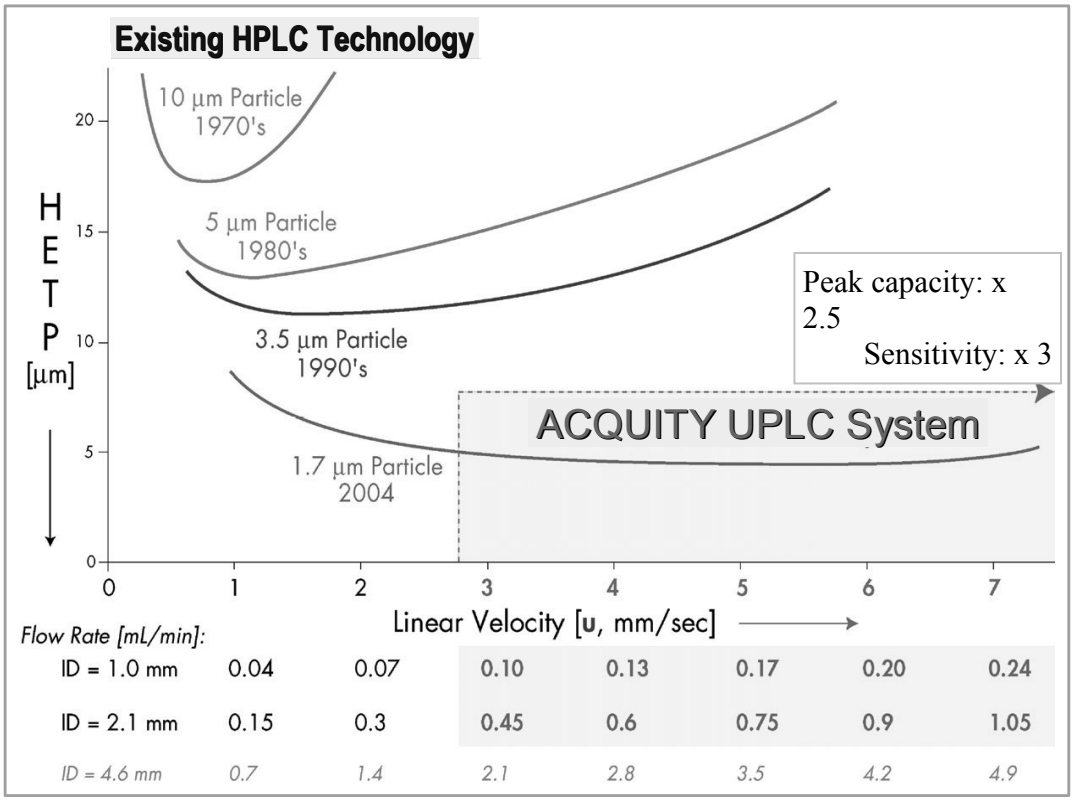

Figure 1. *van Deemter* plot illustrating the evolution of particle sizes over the last three decades (adapted from Swartz & Murphy, 2004)

Materials and Methods

Commercial cheese samples (n = 58) of different types (fresh, soft, semi-hard, hard, very hard) were taken from retail outlets in Vienna, Austria.

Biogenic amines were extracted from cheeses with 0.6 N perchloric acid according to the method described by Novella-Rodríguez, et al. (2000, 2003). Samples were derivatized with AccQ-Fluor reagent (6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate; AQC) according to the Waters AccQ.Tag® precolumn derivatization procedure using the Waters AccQ.Tag® chemistry package (Waters, 1993).

Firstly, analysis of biogenic amines was carried out by a high-performance liquid chromatography (HPLC) method. RP-HPLC was performed on a Waters Chromatography System (Waters Corp, Millford, MA, USA) using a model 600 E multisolvent delivery system, a Rheodyne 7725i injector, guard column and an AccQ.Tag® amino acid analysis column (Nova-Pak™ C_{18}, 4 µm; 3.9 x 150 mm). The fluorescent active derivatives of 18 primary and secondary biogenic amines were separated within 24 minutes. Column eluates were monitored at 395 nm (excitation at 250 nm) using a Waters 474 fluorescence detector interfaced with PC running Waters Millennium^{32} software for automatic quantitation and documentation.

Secondly, the existing HPLC protocol was transferred to ultra-performance liquid chromatography (UPLC™), and an optimized method was established for the simultaneous separation of 20 primary and secondary biogenic amines in cheese. RP-UPLC analysis was performed on a Waters Acquity UPLC™ System using a Acquity UPLC™ column (BEH C_{18}; 1.7 µm; 2.1 x 50mm). The eluted AQC derivatives of biogenic amines were separated within 9 minutes and were detected by monitoring their UV absorption.

According to the method described by Bust et al. (1996), eluent A was 0.005 M sodium acetate solution in 1% tetrahydrofuran (in Milli-Q™ water (Millipore, Bedford, MA, USA). Eluent B was HPLC-grade methanol (Labscan Ltd, Dublin, Ireland). All eluents and derivatized samples were filtered through Millipore filters (HA-aqueous, 0.45 µm, FH-organic, 0.5 µm and GV, 0.22 µm). Samples were analyzed in duplicate, injection volume was 4 µL. Flow rate was 0.4 mL/min, and elution was carried out following the method of Mayer et al. (2006). A standard solution of 20 primary and secondary biogenic amines, containing heptylamine as an internal standard, was used for calibration in the range from 2 to 32 pmol. Concentrations of individual biogenic amines were displayed as pmol, which were subsequently converted to the total amount of biogenic amines calculated as g per kg of cheese.

Results and Discussion

An existing HPLC protocol (Mayer et al., 2006a) was directly transferred to UPLC™ (Figure 2a). Chromatographic conditions were subsequently optimized in several steps (Figure 2b-c) and finally, the eluted AQC derivatives of biogenic amines were separated within 9 minutes by RP-UPLC.

As compared to HPLC, UPLC™ proved to be superior due to shorter run times and higher resolution and sensitivity. UPLC™ takes full advantage of chromatographic principles to run separations using shorter columns, and/or higher flow rates for increased speed. By using smaller particles, speed and peak capacity (number of peaks resolved per unit time) can be extended to new limits (Mayer et al., 2006b).

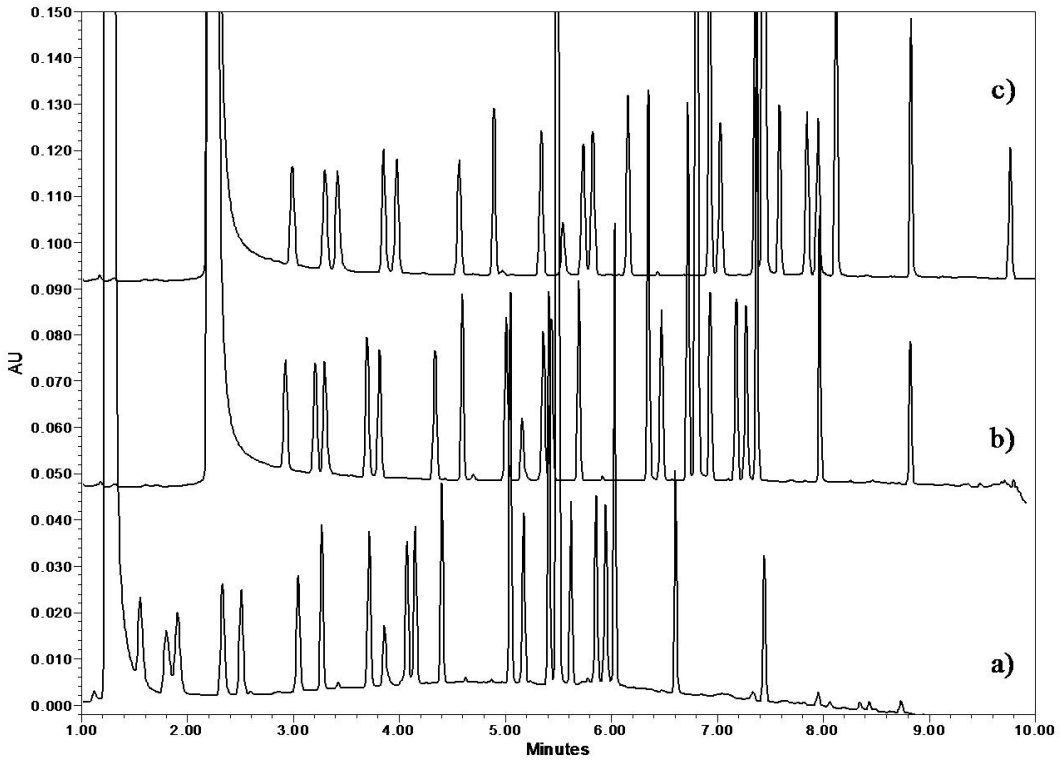

Figure 2. UPLC™ chromatograms of biogenic amines. Optimization of resolution by changing the chromatographic conditions (a – c)

Figure 3 shows typical chromatograms of biogenic amines in a standard solution as separated by HPLC (fluorimetric detection) and UPLC™ (UV detection), respectively. Biogenic amines were separated within 24 minutes using HPLC, whereas only 9 minutes were sufficient for an excellent separation of 20 biogenic amines using UPLC™. Although sensitivity for AQC derivatives of most biogenic amines suffers with UV detection (detection limits are typically 2-20 times worse than with fluorescence detection), UPLC™ analysis showed a higher sensitivity due to a much higher resolution and peak capacity as illustrated in Figure 3. Using UPLC™, peak width at baseline was about 5 seconds; peak weight at half peak height was about 2.2 seconds, which is impressively superior to conventional HPLC systems.

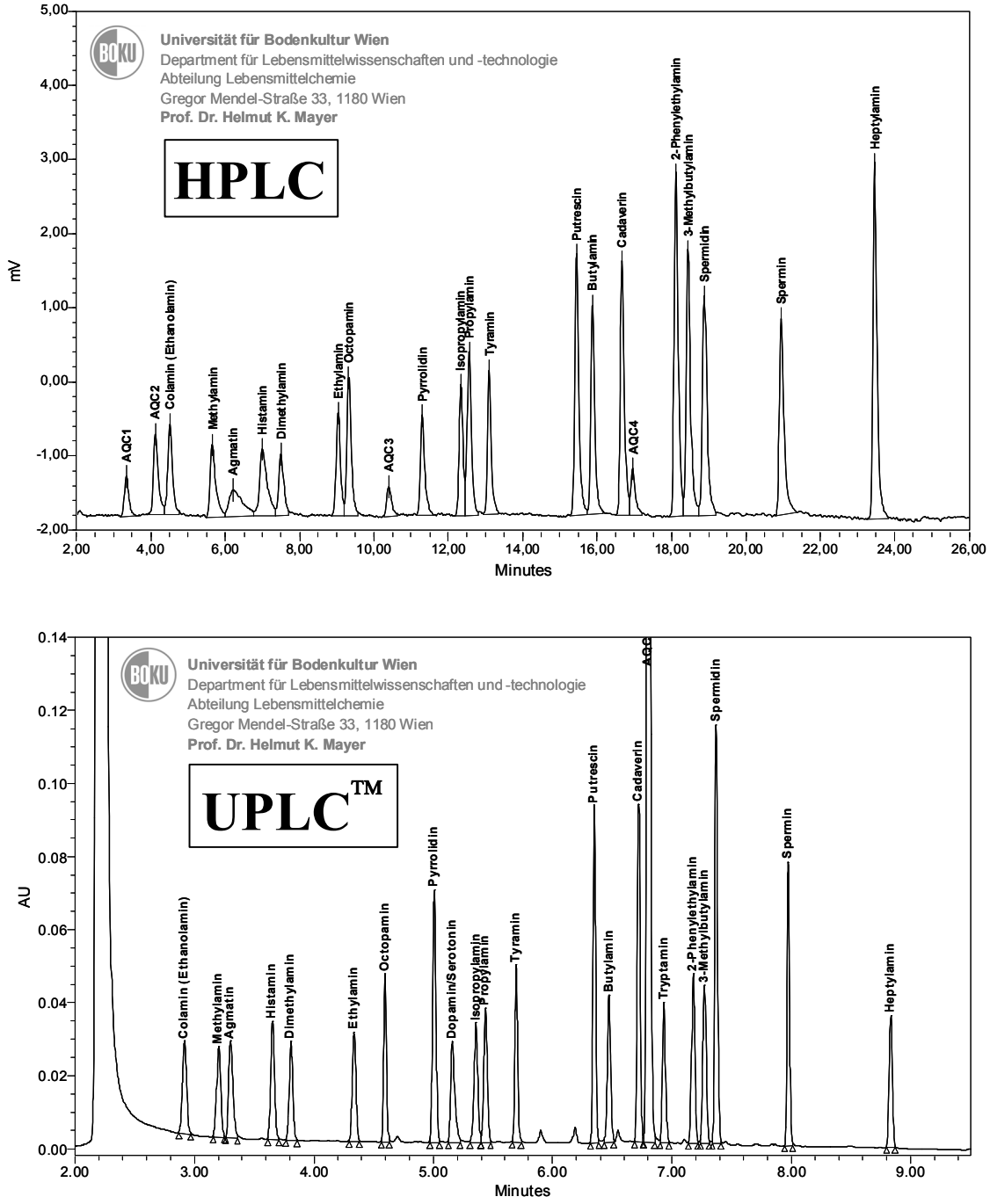

Figure 3. Separation of biogenic amines in a standard solution – HPLC versus UPLC™

Commercial cheese samples from retail outlets in Vienna were analyzed referring to their biogenic amine content using UPLC™. The biogenic amine content varied to a great extent, depending not only on the type of cheese (fresh, soft, semi-hard, hard, very hard), but also within a certain cheese variety.
The highest histamine contents (1160 and 821 mg/kg) were found in two samples of the PDO cheese Tiroler Almkäse (Figure 4), high concentrations also in Vorarlberger Bergkäse (397 mg/kg), a Gorgonzola DOP (255 mg/kg), and a grated Saviola Grana Padano (249 mg/kg). The highest tyramine contents were detected in a sample of Cantal (486 mg/kg), an Olmützer Quargel (480 mg/kg), a Tiroler Almkäse (475 mg/kg), an Appenzeller (375 mg/kg) and an Organic low fat cheese with pepper (354 mg/kg) (Figure 5). The highest putrescine content was found in the same sample of Olmützer Quargel (523 mg/kg), high concentrations also in this Organic low fat cheese with pepper (185 mg/kg) and one sample of Tiroler Almkäse (159 mg/kg). The highest cadaverine content was found in the same sample of Olmützer Quargel (748 mg/kg), high concentrations also in a President leger white mould-ripened cheese (360 mg/kg), in Organic low fat cheese with pepper (306 mg/kg) and a sample of French Camembert (267 mg/100g). The lowest biogenic amine contents were found in Gouda and Edam cheese. Interestingly, long-ripened cheese types as Parmigiano Reggiano and Grana Padano (with one exception) showed very low biogenic amine contents. Most of the Emmental cheese samples tested in this study as well as most of the blue-veined cheese varieties showed also low concentrations of biogenic amines.

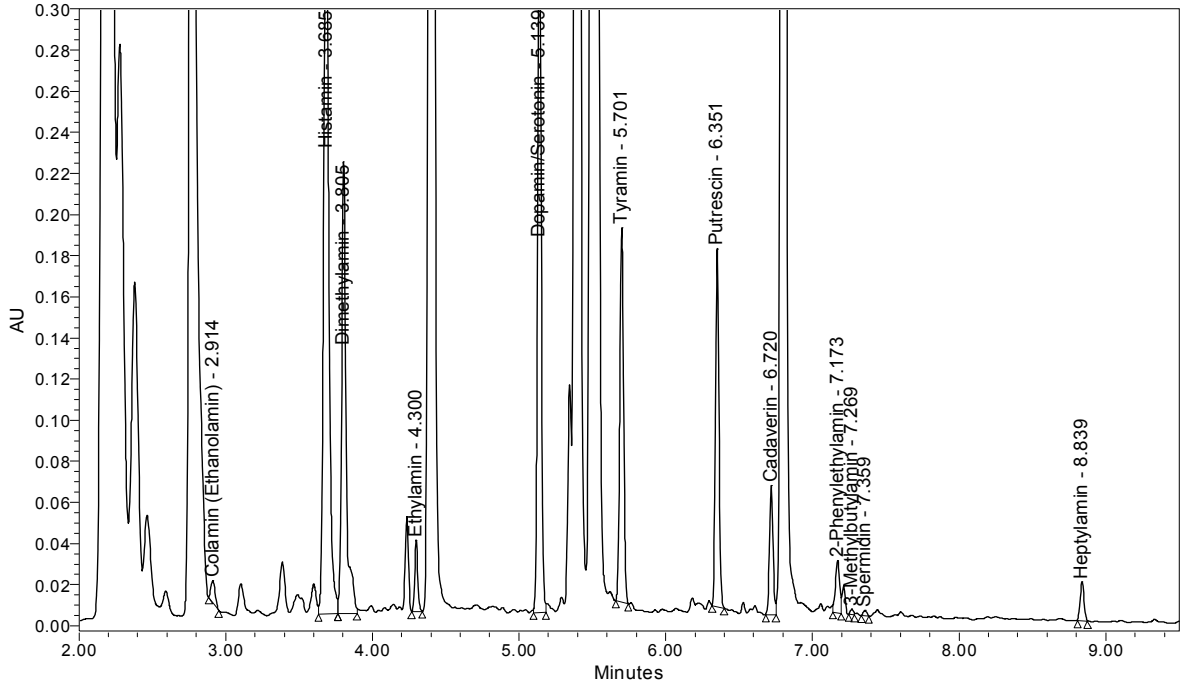

Figure 4. UPLC™ chromatogram of biogenic amines in Tiroler Almkäse (PDO cheese)

The most striking variation in biogenic amine content was observed looking at hard cheese samples of different origin. In one certain sample of Tiroler Almkäse, approximately 1940 mg/kg biogenic amines were found in total, whereas other samples of the same type (e.g., Almkäse, Bergkäse) had a biogenic amine content lower than 100 mg/kg. About 13.8% of samples tested in this study had a histamine content above 100 mg/kg, and 22.4% had a tyramine content higher than 100 mg/kg. Moreover, 8.6% of samples had a putrescine or cadaverine content higher than 100 mg/kg. Thus, the total concentration of biogenic amines in some samples (e.g., Tiroler Almkäse, Olmützer Quargel) was about 1940 mg/kg. These findings are in good agreement with the results found in other studies (Pechanek et al., 1983; Novella-Rodríguez et al., 2000, 2003).

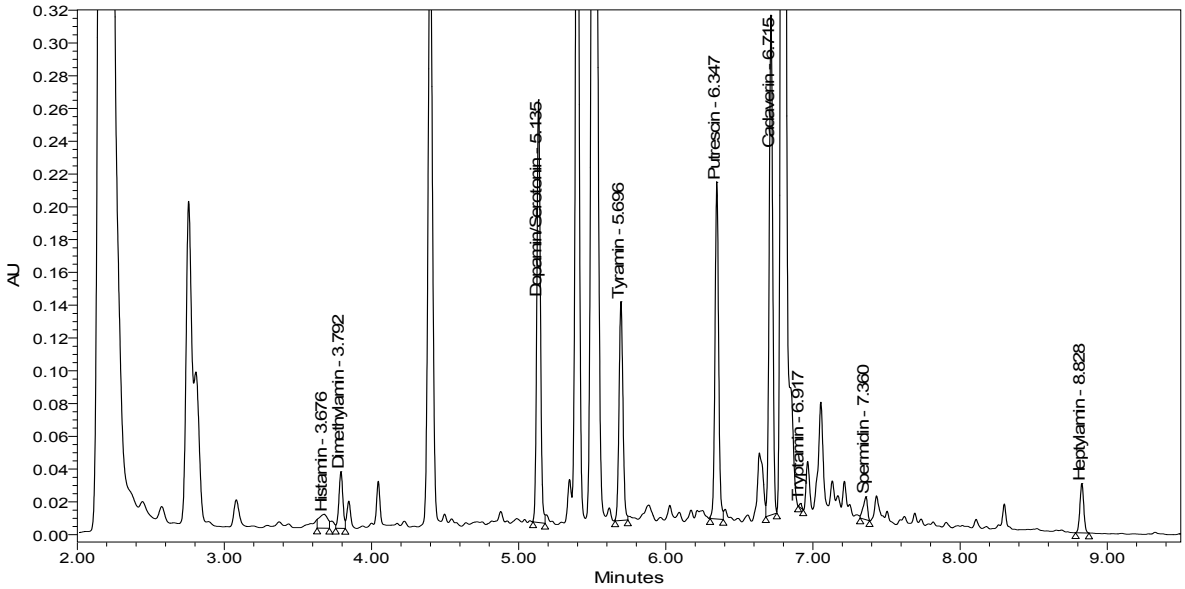

Figure 5. UPLC™ chromatogram of biogenic amines in an organic Low Fat Cheese with pepper

In conclusion, the fast and reliable UPLC™ method proved to be superior to conventional HPLC systems due to shorter run times and higher resolution and sensitivity. As biogenic amine content in cheese samples of different type and origin varied to a great extent, monitoring of biogenic amines (e.g., histamine, tyramine, cadaverine and putrescine) in cheese represents a valuable tool to ensure quality of dairy products in future.

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The development of simultaneously DNA analysis method for rapid ıdentification of soybean and poultry origins in foodstuff

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The addition of soybean proteins and poultry to heat-processed meat products is a common practice mainly for technological and economic reasons. Legislative authority in the most country reported that the companies must accurately label their processed products regarding ingredient content to prohibit the unfair competition between food companies due to the fraudulent substitution or adulteration. Thus, appropriate analytical methods for the detection of food ingredients are essential in order to verify compatible with labeling requirements. This work approaches the dublex determination of additions of soybean proteins and poultry in heat-processed meat products prepared with complex mixtures of meats and by florescence based Real-time PCR.

Appropriate mixtures of poultry and soybean in sausage types were used to develop the assay. DNA was extracted from 25 mg of meat samples using the DNeasy® Protocol provided with the DNeasy® Tissue Kit. Primers specific to soybean *(Glycine max)* and poultry (*Gallus gallus*) were designed for species-specific gene-amplification. Real-time Duplex Polymerase Chain Reaction(Real-time DPCR) was designed for an assay that can combine the advantages of real-time PCR and multiplex PCR to identify poultry and soybean genes more quickly.

Real-time DPCR based on melting temperature (tm) discrimination by using SYBR Green fluorescence dye was developed for the analysis of soybean and poultry contained in mixed meat product. Gene amplicons of poultry and soybean were represented in two melting peaks generated simultaneously at temperatures of 84.3 and 88.8°C respectively. Duplexing results obtained with one of the multiplex polymerase mixes correlated extremely well with the singleplex reference. In this study, a rapid, specific, economical, and accurate real-time DPCR assay was designed by using SYBR Green fluorescence dye that is cheaper than double labeled probes to detect a group of mixed foods simultaneously.

The potential of the described method to detect small amounts of poultry and soybean DNA in industrial meat products may make it a useful tool for inspection programs to enforce labeling regulations of meat products. By the present method, two kinds of the main ingrediend could be identified at the same time more easily and could be widely applied in practical detection for simultaneous identification of other food products if specific primers are designed.

Quantification of beef and chicken proportions in some meat products by real time PCR

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Abstract

Identification and quantification of meat products is becoming a very important issue by avoiding unfair competition among producers, allowing consumers to have accurate information about the acquired products. Identification and quantification of animal species in meat and meat products are also important for the implementation of legislation for food labeling. Identification and quantification of animal species determination are mainly based on DNA and proteins/peptides. Real-time PCR is widely accepted DNA based food analysis method: It is fast, sensitive, specific, easy to handle, reliable and has the potential to create quantitative results.

The aim of this study was to quantify of the animal species in meat products that are sold commercially in Turkey. A total of 30 samples were collected from local market and these samples were analysed for quantification of beef and chicken analyses using Real Time PCR. These samples are kinds of soujouk, sausage, and salami that contain mixture of beef, chicken or turkey species. According to the analyses results some products were found to contain undeclared species that were declared as mixture of the beef and turkey but they contained poultry species. 26 of the 30 samples contained beef species amounts less than declared amounts of the beef species. Results showed that if meat products contain beef, chicken or turkey mixture, beef amounts of these products were less than the declared amount.

Introduction

Food safety, quality, and composition have become the subjects of increasing public concern. Consumers have been given more choices with regard to food composition and dietary requirements via food labels (Aslaminejada et al., 2010).

Authenticity and food composition of food products are also becoming very important issue especially for meat products. Because of the meat products are often composed of more than one meat species. For these products, in a highly competitive market producers use dubious means, which refers to diminished contents of expensive meat, and the substitution of cheaper meats in place of more expensive species, or the use of lower amounts of meat than those are declared on the product, at times the products completely lack the declared meat (Soares et al. 2010, Dooley et al., 2004). Meat species adulteration in ground and comminuted products has been a widespread problem in retail markets (Asensio et al., 2008). In Turkey composed meat products include salami, sausage and Turkish Sucuk (Soudjouk). Authenticity analyses are needed for labeling and assessment of value and is therefore necessary to avoid unfair competition and assure consumers protection against fraudulent practices. Legislative authority establishes that meat products must be accurately labeled regarding species content. (Asensio et al., 2008, Ballin et al., 2009, Sawyer et al., 2003). Some analytical techniques have been suggested for the quantification of meat species in mixed samples, including different protein-based methods such as high-performance liquid chromatography and DNA based method such as Real Time PCR (Bauer et al., 2003, Rodríguez et al., 2005). Methods based on protein analysis have been replaced by DNA-based methods because DNA has the advantage of being a relatively stable molecule and is more able to withstand heat processing (Behrens et al., 1999). Real-time PCR is widely accepted DNA based food analysis method: It is fast, sensitive, specific, easy to handle, reliable and has the potential to create quantitative results.

In this study has been focused on the quantification of the meat species in commercially available meat products such as sausages, salami and Turkish Sucuk (Soudjouk) with DNA based method for determining the adulterations.

Materials and Methods

Materials

The commercial processed foods were purchased from local supermarkets. The samples are different types of meat products such as salami, sausage, Turkish Sucuk (Soudjouk). A total of 30 samples were collected. The samples were stored at -4ºC before the extraction of DNA.

Methods:

DNA Extraction Step

All samples were blended with blender. DNA was extracted from 50 mg of homogenized meat samples using R-Biopharm Sure Food DNA Extraction Animal X kits following the manufacturers instructions. Briefly, 50 mg samples were incubated with lysis buffer and Proteinase K at 65ºC for 30 min. After the centrifugation 0f sample lysate, Binding buffer were added to liquid supernatant for the DNA binding on the silica membrane. Spin filter were washed with wash buffer for the DNA purification. The last step is DNA elution from the spin filter with elution buffer. All DNA extraction was done in duplicates. The purity of genomic DNA was checked by using spectrophotometer taking O.D.260–280. PCR inhibition reaction were tested using positive control.

Real Time PCR Step

For quantification of beef and chicken from meat products, R-Biopharm Sure Food kits were used. These kits are for beef-DNA relative to the total animal DNA content. Therefore the kit contains two PCR systems (two kind of master mix), one for detection of beef or chicken specific gene and one for the detection of an animal gene. After the preparation of master mix and sample DNA were processed in Real Time PCR. For each reaction, extraction control, positive control and negative control were used to prevent wrong results. The reactions were run in duplicate in a reaction volume of 25 µL, too.
Amplifications were carried out in a Mastercycler ep gradient S termocycler (Eppendorf).

Table 1. Chicken Quantification Real Time PCR Setup

Program	Eppendorf Blockcycler
Initial Denaturation (HOLD)	5min 95◦C
Denaturation	15 sec 95◦C
Annealing/Extension	30 sec 60◦C

Table 2. Beef Quantification Real Time PCR Setup

Program	Eppendorf Blockcycler
Initial Denaturation (HOLD)	5min 95◦C
Denaturation	10 sec 95◦C
Annealing	15 sec 62◦C
Extension	30 sec 65◦C

The Real Time PCR was programmed for a 45 cycle PCR that was composed of denaturation and annealing/extension step.

Results and Discussion

DNA is a relatively stable molecule and has been successfully extracted from meat products. According to the UV-spectrophotometer results, high-quality DNA were extracted from the samples. In this study, 30 meat samples were analysed for the quantitative detection of beef and chicken.

8 of the samples are soudjouk, 8 of the samples are salami and 14 of the samples are sausages. 20 of the samples were contained beef and chicken, 10 of the samples were contained beef and turkey. According to the analyses results, meat from undeclared species was found in 20% of cases. Primarily with chicken substituting turkey and beef species. Because chicken species is more cheaper than turkey or beef species.

Totally 26 of the 30 samples contained beef species amounts less than declared amounts of the beef species. Results showed that if meat products contain beef, chicken or turkey mixture, beef amounts of these products were less than the declared amount.

The results of these examinations indicate that, on meat product labels was incorrect in more than %80 of the cases.

Conclusion

The importance of the quantitative detection of meat species in meat products is increasing. Because of these, the analyses methods should be reliable, sensitive and accurate. Real Time PCR is the primary tool for screening, qualitative and quantitative meat species analyses. Using Real Time PCR a determination of the percentage of an animal species relative to the meat total. On the basis of our results, quantitative meat analyses are requisite all of the meat products to provide to avoid unfair competition.

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Consumer Trends and Food Industry Innovation Highlights

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Consumer researches on the consumer needs indicate the life gols as; Achieve more, Look better, Feel better daily, Be healthy for longer, Be free from health problems and Give kids a good start.

Consumer Trends are the key of innovation roadmap. Trends guide industry to anticipate the changes in consumers' lives thus able to prepare for the future by thinking more innovatively in developing the product brands.

Trends are a ready source for stimulus in developing ideas for products, promotions and communications.

Weakening of traditional values, Globalization (of tastes, people & trade), Advances in science & technology, Rising affluence , Increased access to information, goods and services, Economic, social, political and environmental changes are shaping people's attitudes, values and behaviours at a global and local level.

The reports of the Market Research companies on the basic lifestyles and trends have been evaluated and summarised in this paper.

The Food Category trends are explained in three steps.

At the first stage the basic human trends are mentioned. These are summarised as Vitality (stimulating consumers while maximizing the human potential), Recognition (uniqueness, to be different), Control & Complexity management (data driven), Belonging (true and honest personal relationship), Conviviality & Getting Connected (social togetherness) and Enjoyment & Pleasure Hunt (seeking or giving pleasure).

In the second step the preferences and trends in Food Health and Nutrition are highlighted. Health & Wellness trend is located at the heart of the consumer trends. The main trends around Health and Wellness are explained with examples from the market. Functional Foods addressing Digestive Health and Energy are the trends having growth opportunity. Naturally healthy in other words health benefit of a food or an ingredient combined with convenience is still high in the agenda.

In the later stage the food product trends in connection with the basic human trends have also been interpreted, again with the examples from the market. The leading ones are Indulgence with the Health Benefit, Nostalgic, Exclusivity, Convenience to fit the life styles and Streamlined communication.

Packaging innovation is still a key driver of success among all food categories.

One of the important trend and increasing consumer preference is Care for Environment and Social Corporate Responsibility & Mission.

In conclusion from the evidences in the market it is a fact that successful innovation often comes from continuous monitoring of the consumer trends and combining trends together in concepts and brands, rather than looking at them in isolation.

Plasma-a novel food processing technology

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An increase in the world population has brought about rapid change in the field of food technology and the pace of change is ever increasing. As a result, major motivations that aim to determine new, emerging or future food technologies are developing. This trend signifies the response of food science and industry to the demands of consumers. The increasing awareness of food related health risks and changing lifestyles have resulted in expectations for fresher, more natural foods, which are less severely processed, contain less, preservatives or are even free from "artificial" additives and are nutritionally more advantageous. In the search for less severe or 'minimal processing' technologies new, 'non-thermal' methods of food preservation are taking the lead. Mild preservation technologies enhance the shelf life of foods and have a minor impact on the quality and fresh appearance of food products. Perhaps the most novel of all ‘non thermal’ technologies is non thermal plasma (NTP’s) also referred to as cold atmospheric plasma. Like any novel technology NTP’s at normal atmospheric pressure were originally developed for a certain use (delicate electronics) and has since been applied to many fields such as medical devices, biomedical, textiles and dental applications. More recently, NTP’s are being investigated for their application to foods and beverages. Studies on NTP’s have shown it to injure and inactivate pathogenic bacteria on inert food contact surfaces, on some solid foods and in liquids. The future prospects of NTP technology are great for application to the food industry.

Effect of ultrasound and temperature on enzyme activity and quality indicators of fresh Iranian lime juice

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Abstract

The most heat stable enzyme in lime, lemon and other citrus juices is pectinmethylesterase (PME). This enzyme induces pectin destabilization, which causes cloud loss in the juice. The cloud presents the fresh-like property and therefore product satisfaction. Inactivation of PME is generally used as an indicator of the adequacy of pasteurization because it is known to be more heat resistant than the common micro-organisms. Ultrasonic treatment is one of the emerging tools that could be the alternative to thermal processing. It can enhance convective heat transfer as well as generate bubble explosion, which produce local hot spot that can cause micro-organism and enzyme destruction. However, ultrasonication (US) alone cannot inactivate the thermo-stable PME, even at long exposures. The combination of ultrasound and heat (thermosonication, TS) can slightly decrease the activity of this enzyme, which depended on time and temperature.

Key words: Lime, thermosonication, pectin methyl esterase

Introduction

Commercial lime and lemon juices are among the world most important citrus products. For example, these juices are used as a common ingredient in most of the traditional Asian cooking. Moreover, they are necessary for many global food productions, such as lemonade drink, marmalade, jams, candies, jellies, desserts, pharmaceutical products, and medicines. Lime juice, itself, is a colloidal suspension of cellular and polymer particles. This cloudy appearance is an important property of the juices since it gives the natural appeal of the fresh juices. Colloidal stability is maintained by pectin molecules through a complex and not well understood mechanism. Cloud loss of citrus juices is an intensively studied problem in food technology. It is due to the action of endogenous pectin methyl esterase (PME) on pectin substance. PME catalyzes the de-esterification of pectin molecules. De-esterified pectin molecules are able to interact through calcium bridges, leading to cloud loss and phase separation in single-strength lime juices and gelation in their concentrates. Stabilization of cloud in citrus juices requires the inactivation or inhibition of PME (Vercet, et al., 1999). Several strategies have been used to inhibit or inactivate PME avoiding the negative effects of intensive heat treatments. Inhibition of PME by polyphenols (Hall, 1966; Pilnik and Voragen, 1991), inhibition by specific proteic PME inhibitors (Castaldo et al., 1991), or inhibition by the oligogalacturonides produced by the action of added polygalacturonase or pectinylase (Baker and Bruemmer, 1972; Krop and Pilnik, 1974; Termote et al., 1977) have been suggested as alternatives to the heat treatments. Other strategies rely on PME inactivation by non-thermal treatments such as high pressure (Irwe and Olson, 1994; Donsi et al., 1996; Cano et al., 1997; Knorr, 1998), low pH values (Owusu-yaw et al., 1988), or supercritical carbon dioxide (Balaban et al., 1991). Another possible alternative is ultrasound in combination with heat (Thermosonication, TS). The aims of the study were to investigate the effect of ultrasound on the inactivation of lime pectin methyl esterase and juice quality. One further aim was the evaluation of the kinetic parameters of the TS effect on lime pectin methyl esterase.

Materials and Methods

Preparation of lime juice

Whole Iranian limes were washed and juice was extracted manually. The juice was then filtered using cloth. The filtrate was centrifuged at 4000 rpm (r=15 cm), at ambient temperature for 6 min to remove pectin.

Equipment and experiment set-up

A submerged ultrasonic horn for 100 ml, with a tip diameter of 7 mm and fixed frequency 30 kHz, actual power 100 W and amplitude 100% was used. Experiment performed on hot plate stirrer adjusting temperature to 40°C-90°C. One thermometer was placed in solution to observe the temperature profile. Time was started measuring as soon as the temperature of the solution reached the water bath temperature.

Thermosonication

Heat treatments were performed between 40°C - 60°C. The ultrasound pulse cycle was adjusted to 20% to perform the interval reaction.

Pectin methyl esterase (PME) activity assay

PME activity was measured using the method described by Kimball (1991). Ten millilitre of lime juice was mixed with 40 millilitre of 1% pectin-salt substrate. The solution was adjusted to pH 7.0 with 2.0 N NaOH, then the pH of solution was re-adjusted to pH 7.7 with 0.05 N NaOH. After the pH reached 7.7, 0.10 millilitre of 0.05 N NaOH was added. Time was measured until pH of the solution regained pH 7.7. PME activity unit (PEU) and the relative PME activity (%) were calculated by the following formula (Kimball 1991):

$$\text{PEU} = \frac{(0.05\ \text{N NaOH})(0.10\ \text{mL NaOH})}{(10\text{-mL sample})(\text{Time in min})} \quad (1)$$

$$\text{Relative PME activity (\%)} = \frac{\text{PEU of treated lime juice} \times 100}{\text{PEU of control lime juice at d0}} \quad (2)$$

Total dry solids dissolved in the juice (Brix value)

The measurement of dry substances was carried out by refractrometry (CARL ZEISS).

pH value

The pH of the juice was measured through the pH electrode (LABTRON, PHT 110) at 20°C.

Evaluation of *D* and *z* values of enzyme for thermal process calculation

As in thermo-bacteriology, enzymatic inactivation features kinetic parameters that make it possible to compare the degree of inactivation and the thermal treatment defined by the temperature-time curve. The most important kinetic parameter is the decimal reduction time (*DT*) and its dependence on temperature expressed by *z*. The decimal reduction time was calculated according to Stumbo (1973) by equation:
$DT = t/(\log A_i - \log A_f)$ (3)

Where *Ai* is the starting activity; *Af* is the residual activity that survives the thermal treatment; and *t* is the thermal treatment time at temperature *T* in minutes.

The *z* parameter was derived from *log DT* values at different treatment time versus temperature. The *z* parameter indicates how many degrees the temperature must change for the decimal reduction time to be 10 fold higher or lower. $z = -(T - T_{ref})/(\log D - \log D_{refT})$ (4)

Where T = Temperature in °C (or K) for the lower temperature

T_{ref} = Temperature in °C (or K) for the higher temperature

D = *D*-value for the lower temperature (min)

D_{refT} = *D*-value for the higher temperature (min)

Statistical analysis

All experiments were run at least twice, and analyses of all samples were run triplicate and averaged. Statistical analyses were carried out using SPSS software. Significance of differences was defined at $P< 0.05$.

Results and Discussion

Temperature dependence of PME inactivation

The influence of thermal treatment was investigated by the inactivation of lime pectin methyl esterase under standard condition of pH and reaction time. In the temperature range between 40 and 50°C, the activities of PME dropped slightly in one hour treatment (Figure 1 and 2). However, the inactivation of enzyme was increased at temperature above 60°C. Accordingly, the residual activity was approximately 7.954% after heating at 80°C for 1 min and 2.323% after heating at 90°C for approximately 1 min. t=0 for all graphs indicates the moment of reaching lime juice temperature to necessary temperature. In Table 1, *D*-values were estimated from the plot in Figure 3 and 4, where *z*-value was obtained from *D*-value plot (Figure 5). MacDonald et al. (1993) identified seven fractions of lemon pectin methyl esterase, which considered being the heat sensitive and the heat stable fractions. Two major heat stable PME were found in peel and endocarp separately. These enzymes were completely inactivated at temperature above 88°C (MacDonald et al., 1993). In this study, the heat sensitive fractions were rapidly inactivated at 60°C. The *z*-value of heat treatment was calculated to be 40.7°C. The *z*-values obtained earlier by some researchers for PME of orange, mandarin and grapefruit were 11, 11.4 and 5.2 °C, respectively (Eagerman & Rouse, 1976; Nath & Ranganna, 1977; Versteeg, 1979). In addition, the *z*-value of orange juice pulp PME was estimated to be 6.5 and 10.8°C for the sensitive and stable fractions (Wicker & Temelli, 1988).

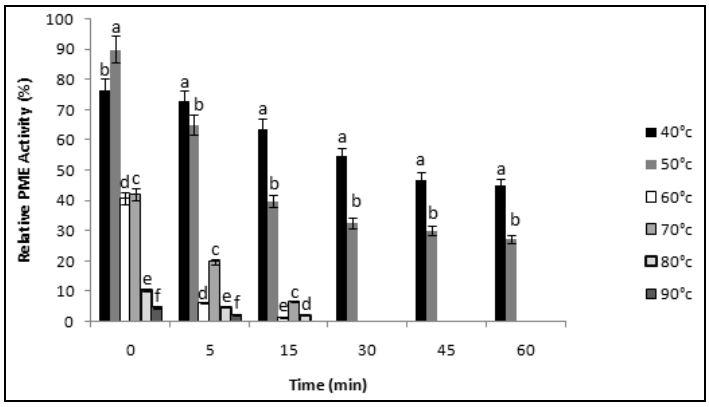

Figure 1. Relative PME activity (%) at various temperatures in 60 minutes of continuous heating

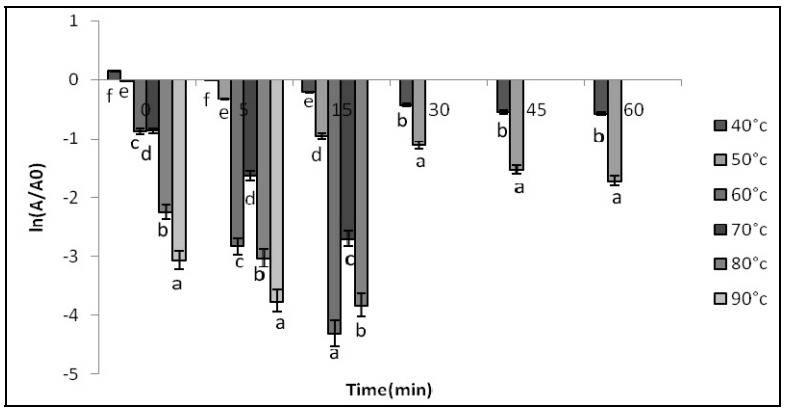

Figure 2. Inactivation of PME at various temperatures in 60 minutes of continuous heating

Decimal reduction time of PME inactivation

The PME activity decreased with increasing time of exposure (Figure 1 and 3).
To evaluate the effect of treatment time, the decimal reduction time (*D*-value) was calculated and presented in Figure 5.

It has been found that the *D*-values at each temperature show significant differences (Table 1). The *D*-value at 90°C was 3.30 min. Eagerman and Rouse (1976) also reported processing orange juice at 90°C for *D*-value of 30 sec was enough to stabilize the cloud. Versteeg (1978) demonstrated a *D*-value of 22 sec at 90°C is necessary to inactivate the heat stable PME isozyme. In this study, *D*-values for PME inactivation ranged from 3.30 min at 90°C to 55.92 min at 40°C (Table 1). However, the *D*-value of the experiment at 90°C was greater than the values from Eagerman and Rouse (1976), and Versteeg (1978).

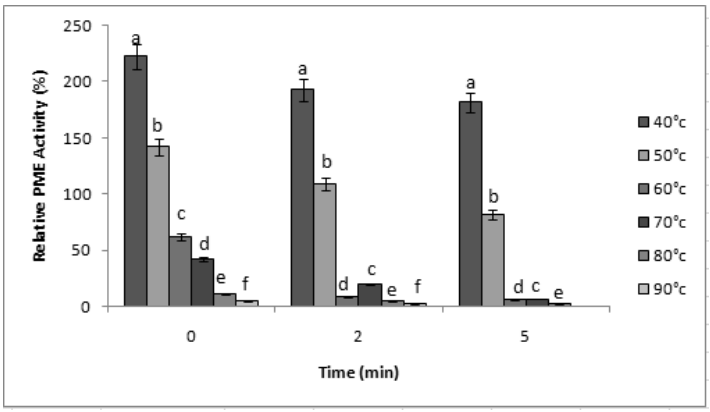

Figure 3. Relative PME activity (%) at various temperatures in 5 minutes of continuous heating

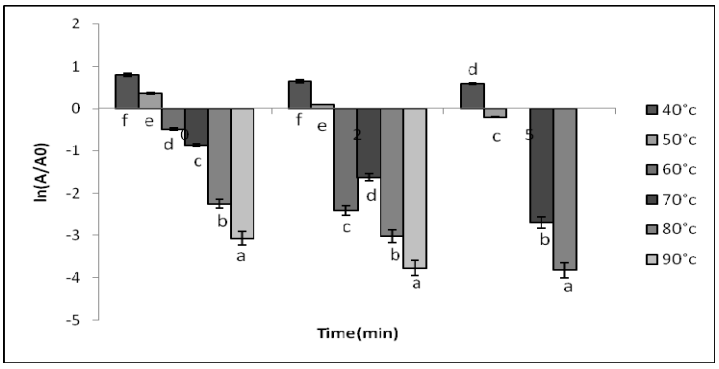

Figure 4. Inactivation of PME at various temperatures in 5 minutes of continuous heating

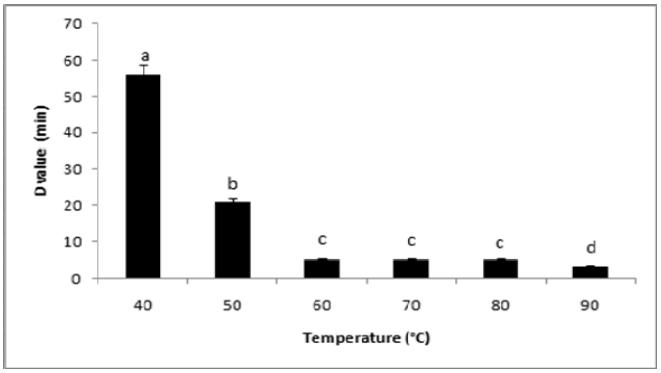

Figure 5. *D*-Value of PME inactivation at various temperatures

Table 1. *D*-values of heat and thermosonication PME inactivation

Temperature (°C)	*D*-value of heat treatment (min)	*D*-value of Thermosonication treatment (min)
40	55.92815	27.67587
50	20.86457	22.32774
60	10.32121	4.551167
70	5.030722	---
80	5.019853	---
90	3.30521	---
z- value(°C)	40.70231	25.51091

Discussion on thermosonication inactivation of lime PME

It was suggested to combine the ultrasonic treatment with temperature (thermosonication) to increase the inactivation rate (Kuldiloke et al, 2002). The experiments were set up by applying ultrasound 30 kHz with various temperatures for different periods (figure 6 and 7). The temperature of the PME suspension remained constant during the experiment. Actually, the temperature varied at ±2°C with respect to the average temperature.

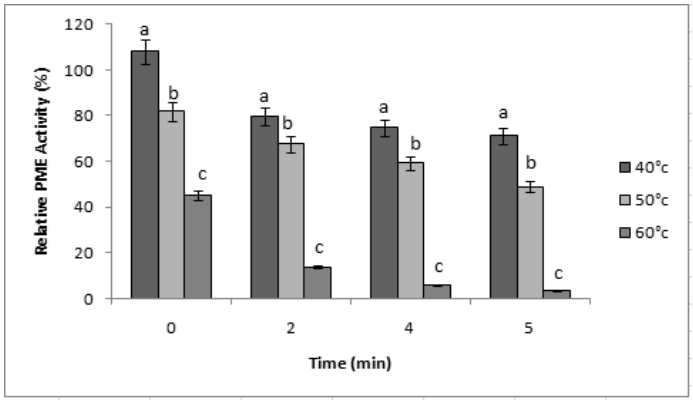

Figure 6. Relative PME activity (%) at various temperatures in 5 minutes of thermosonication

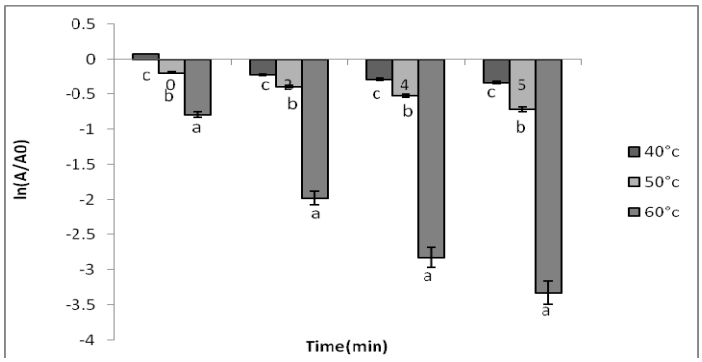

Figure 7. Inactivation of PME at various temperatures in 5 minutes of thermosonication

Brix and pH

Brix and pH of fresh lime juice was calculated about 8.5 and 2.47, respectively.

Conclusion

The inactivation of pectin methyl esterase by ultrasonic wave is dependent on time of exposure and temperature. Ultrasonic wave propagation through a liquid creates cavities (bubbles), mainly in weak

spots created by dissolved gas molecules. Once a critical size has been reached, the cavity grows rapidly until it can no longer sustain itself and then collapses (cavitational collapse). The liquid stream produced in the vicinity of the bubble, either by bubble oscillation or implosion, often results in high temperature that can promote protein denaturation. In this aspect, increase in pressure can enhance the bubble implosion. Pressure and temperature have been taken into account to promote the chemical reactions involving free radicals that are formed by the decomposition of water inside the oscillating bubbles and lead to the inactivation of enzyme and micro-organism. Correspondingly, the ultrasound wave can also enhance this thermal and pressurize process since the ultrasound itself can improve the fluid-to-particle convective heat transfer (Kuldiloke, 1995). Among these mechanisms, (pressure enhancing US, US enhancing heat and Heat & Pressure inducing chemical reaction), inactivation of the enzymes and destruction of microbial cells can be achieved.

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A novel technique for particle generation with air-assisted sub-critical water extraction technology

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Abstract

Natural extracts are widely used in groceries, pharmaceutics and nutraceuticals. Conventionally, these extracts have been obtained by water or organic solvent extraction. In the last few years, several supercritical fluid-based techniques have been proposed for the production of micronic and nanometric particles. These processes utilize several specific properties of gases at supercritical conditions, particularly enhanced solubilization power and its modulation, large diffusivities, solventless or organic solvent reduced operation, and the connected possibility of controlling powder size and distribution. Currently, three common routes for particle formation in supercritical fluids are available: rapid expansion of supercritical solutions (RESS), supercritical anti-solvent process (SAS) and particle generation from gas-saturated solutions (PGSS). As each route is different, in this work, a new design utilizing an innovative process is presented allowing a combined extraction mechanism, together with particle production, both being novel developments. The process is based on a combined extraction and particle production technique. The basic principle is rapid depressurization of saturated solutions after extracting with water, and the precipitation of the solution results in the form of powder. The mushroom *Ganoderma lucidum* was used as sample material. Powder quality was studied based on temperature and pressure effects, and particle size profile was investigated. Also, the extract composition was identified together with molecular size determination.

Introduction

There is an increasing public awareness of the health, environment and safety hazards associated with the use of organic solvents in food processing and the possible solvent contamination of the final products [1, 2]. The high cost of organic solvents and the increasingly stringent environmental regulations together with the new requirements of the medical and food industries for ultra-pure and high added value products have pointed out the need for the development of new and clean technologies for the processing of food products [3-9].

Supercritical fluid technology will allow pharmaceutical and nutraceutical companies to develop products of standardized concentration of active ingredients, and will simultaneously produce nutraceutical and pharmaceutical products of much higher concentration (higher yields and purity) and quality (with less creation of artifacts), than possible by conventional chemical engineering unit operations, such as liquid/liquid extraction, distillation, mechanical micronization, liquid and/or gas phase reactions, etc[10-15].

The originality of this research lies on the originality of design and the new alternative experimental technique. In order to determine this novel methodology, a system was designed having combined properties of extraction and particle generation. The basic principle involved in the production of extract particles is rapid depressurization of saturated solutions after extracting with sub-critical water, and the precipitation of the solution in the form of powder.

Materials and Methods

Materials and chemicals

Ground form of dried *Ganoderma lucidum* in 3 mm of particle size was used as working material and it was supplied by Refarmer Co., Ltd., Japan. Ganoderma contains 6.52 % hydrogen, 1.20 % nitrogen, 50.26

% oxygen and 42.02 % carbon (values are given by Kumamoto University Elemental Analysis Center, Kumamoto, Japan). Ganoderma samples were stored at room temperature keeping away from humidity until they used and did not undergo any further pretreatment. Polystyrene standard kits were acquired from Shodex Company (Shodex STANDARD S Series- SM 105, Shodex Ltd., Tokyo, Japan). Tetrahydrofuran (THF) was purchased from Wako Chemicals (Japan) GPC solvent. HPLC-grade solvents including acetonitrile (CH_3CN) and acetic acid (CH_3COOH) were purchased from Wako Chemicals (Tokyo, Japan).

Digitization of a video or electronic image captured through an optical microscope (Nikon Eclipse E200, Tokyo, Japan) was done to investigate the particle distribution and average size depending on temperature. Particle structures of the powder microcapsules were evaluated by JEOL JSM-5200 model (Tokyo, Japan) scanning electron microscope. The samples were systematically observed with 1500 of magnification. The average diameter of aqueous droplets was determined by the dynamic laser scattering (DLS) method using SALD-ER laser diffraction particle size analyzer (Shimadzu Co., Ltd., Japan). For determination of constituents of generated powders, an analytical system was developed using HPLC with an ODS column. The mobile phase was composed of 1 % AcOH/H_2O-CH_3CN and 2 % AcOH/H_2O-CH_3CN, and the elution profile was monitored at 243 and 250 nm for ganoderma alcohols and acids respectively. Molecular weight distributions of extracts were determined via size-exclusion chromatography (SEC) equipped with a UV-visible detector (UV-970, Jasco Co., Japan) and TSK Gel GMHXL-L column (7.8 mm (ID) × 30.0 cm (L); Tosoh Co., Tokyo, Japan) using a calibration curve derived from polystyrene standards of known molecular weights.

Design of a novel system: combined extraction and air- assisted particle formation system

Apparatus is designed for experiments in temperature range from 25 to 200 °C and pressures up to 10 MPa. The extraction vessel placed in the oven was heated to the extraction temperature and accurately controlled to within range of 2 °C difference. This novel technique is a kind of rapid expansion technology that is used for particle production processing. In order to combine the extraction technique with particle formation process, a system was designed being divided into three main sections such as;

- A supercritical fluid delivery unit (a water source, a circulating heater, an HPLC pump) (Figure 1.a.),
- A solute dissolving and extracting unit (a pre-heater, an extraction vessel and an oven) (Figure 1.b.), and
- A crystallizing-separating unit (nozzle and collector) (Figure 1.c.).

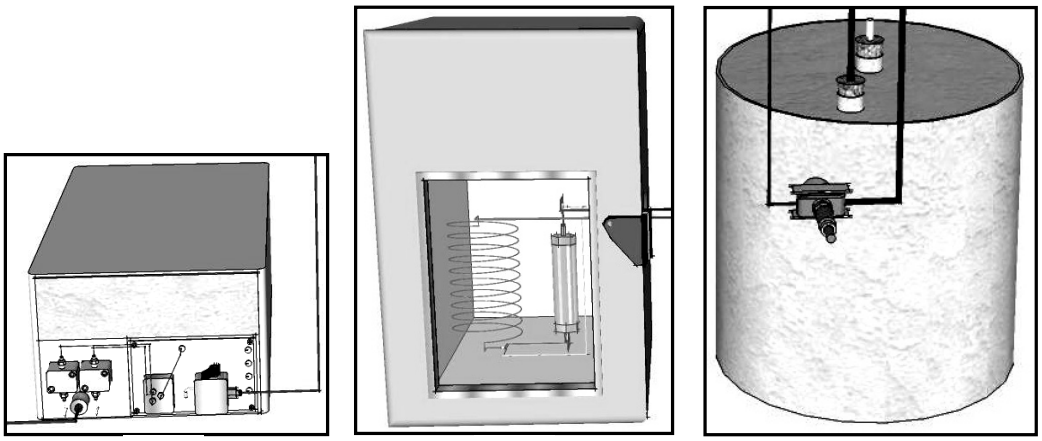

Figure 1. Design components of combined system; (a) sub-critical fluid delivery unit, (b) solute dissolving and extracting unit and (c) crystallizing-separating unit.

In the particle generation section, the nozzle was inserted to the edge of collection chamber at a specific angle. The appropriate angle was calculated by considering technical properties of air atomizing nozzle.

In order to create a cyclic flow, hot air stream was fed to the chamber from a specific distance to encourage the circulation. A ventilation exit was placed on the chamber to prevent condensation that was occurred as a result of insufficient saturation of dew point of air stream and to evaporate the moisture content of air. Air atomizing nozzle produces a fine atomization by mixing air and liquid. Each nozzle in the series is available with a pressure spray setup. Plus, spray setups, within the series, are interchangeable for application flexibility. They are also offered with internal or external mix and atomization control.

Experimental procedure

A schematic representation of the experimental apparatus was used to produce *Ganoderma lucidum powder is* shown in Figure 2. This system has a novel property of being a combination of two different chemical operation systems of extraction and particle generation. It makes us save energy, time and money with products being better in quality.

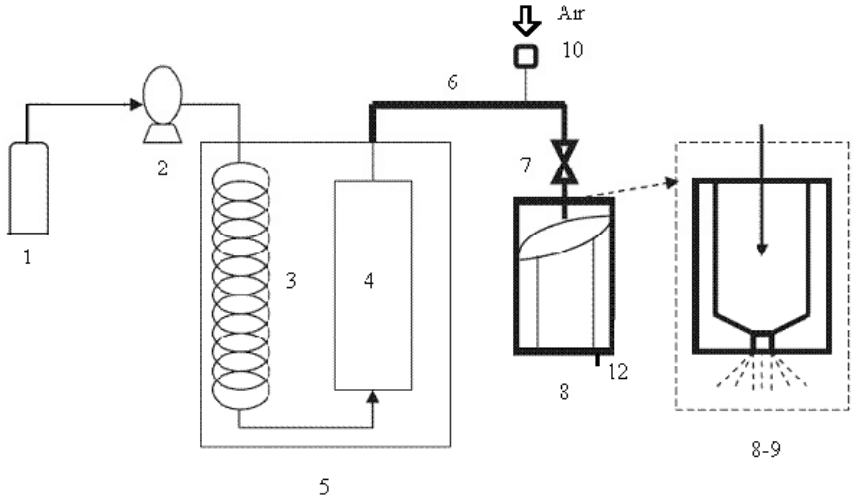

Figure 2. Schematic illustration of experimental apparatus: (1) Distilled water reservoir ; (2) HPLC pump; (3) pre-heater; (4) extraction vessel; (5) oven; (6) extract outlet pipe line; (7) ball valve; (8) collection chamber; (9) air atomizing expansion nozzle; (10) air inlet-needle valve; (12) vacuum-air exit.

In this study, two major types of flow mechanisms have been designed. In the first one, after the extract passed through the outlet pipes, the air stream was given in counter flow to the extracted liquid stream. In the second, concurrent flow was applied. After the extract outlet pipe, air and extracted solution were fed to an air atomizing nozzle in which those two streams were mixed inside the nozzle.

Particle formation experiments were performed at three different extraction (pre-expansion) temperatures (T_o= 100, 150, and 200 °C) and two different extraction pressures (P_o= 5 and 10 MPa). These pre-expansion and extraction conditions were selected to prevent particle precipitation inside the capillary nozzle (J series round spray, Spraying Systems Co., Wheaton, USA), and subsequently clogging the nozzle. The extraction temperatures and pressures equaled respectively to the pre-expansion temperatures and pressures in all experiments. Air pressure and water flow rate were kept constant at 0.5 kPa and 0.5 mL/min, respectively.

Results and Discussion

Experiments were carried out: at P_o=5 and 10 MPa, at T_o= 100, 150 and 200 °C, T_{air} = 175 ~ 180 °C. In experiments that were done in counter flow, there were some plugs in the air-extract exit and extracted sample material got stuck in outlet and some burnings occurred. In this mechanism, the counter flow could not form spray effect and instead of powders, only thin film formation, sometimes slurry was occurred being the most efficient at 200 °C.

Investigation of counter flow mechanism showed that there are some handicaps in formation of particles because of flow mechanism in the system design. So, the system was modified and nozzle was installed in order to spray the extract solution in air assistance. Particle formation was observed around the edge of nozzle and at the bottom of collecting chamber. Amount of powder was negligible at 100 °C. More amounts were produced at higher temperatures, being mot at 200 °C.

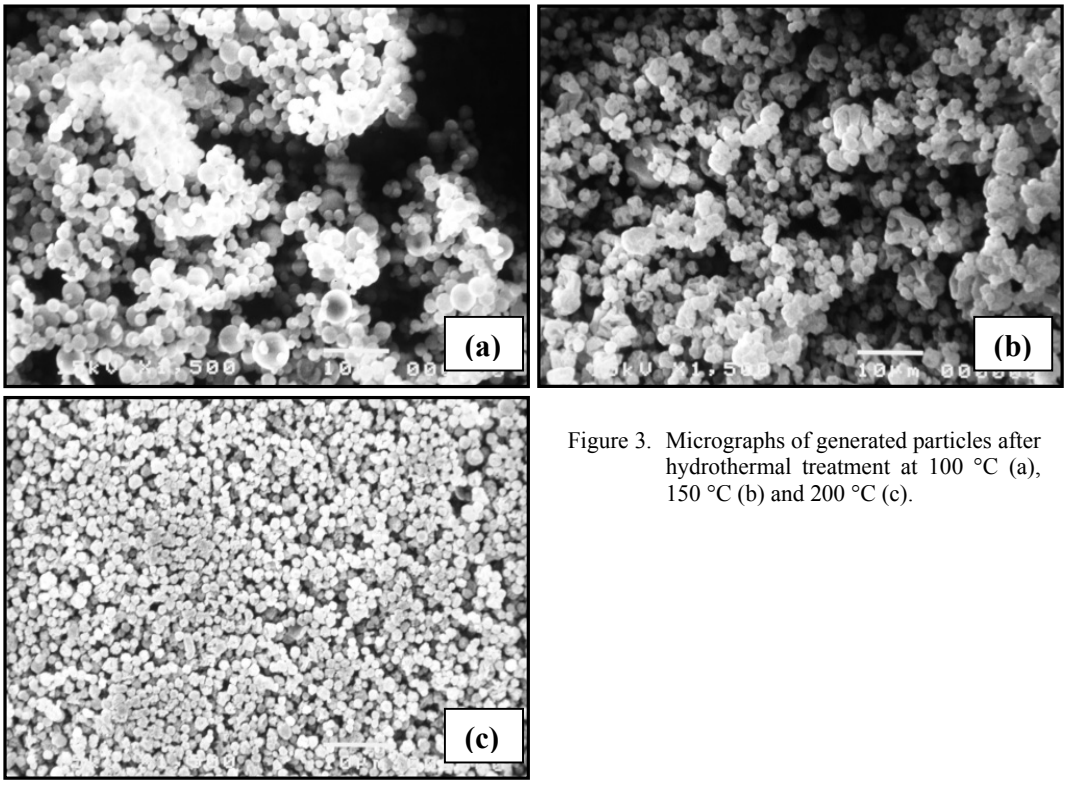

Figure 3. Micrographs of generated particles after hydrothermal treatment at 100 °C (a), 150 °C (b) and 200 °C (c).

Figure 3 shows SEM pictures of precipitated ganoderma powders, which are almost spherical and mostly have a size of about 3–5 μm, formed at 5 MPa extraction pressure and extraction temperatures of 100, 150 and 200 °C with air atomizing nozzle (Spraying Systems Co., Wheaton, USA). The particles were collected at the bottom of collection chamber. The increase of the pre-expansion temperature from 100 °C to 200 °C produced a large decrease of particle size and a narrowing of particle size distribution. At pre-expansion temperature of 200 °C very small particles with mean particle size about 4 μm were obtained. With respect to temperature effect, it can be seen that for the same pre-expansion pressure and other processing variables, a higher extraction temperature means a smaller size of the obtained particles. It might be due to favorable dissolution of reishi in sub-critical water at specific temperature. Higher temperature leads to higher super saturation and higher nucleation speed, and then more crystal nucleus. Smaller size particles are formed consequently.

The average diameter of aqueous droplets was determined by the dynamic laser scattering (DLS) method using SALD-ER laser diffraction particle size analyzer (Shimadzu Co., Ltd., Japan). Prior to the analysis powders, whose particle size was bigger than 100 μm were separated using molecular sieves in 100 U.S. mesh size. 18 % of powders were greater than 100 μm and 1 % of particles were smaller than 0.001 μm. For the 81 % part of sample particles, DLS method was applied and it was seen that higher temperatures gave narrower distribution in smaller particle size as this phenomena is shown in Figure 4.

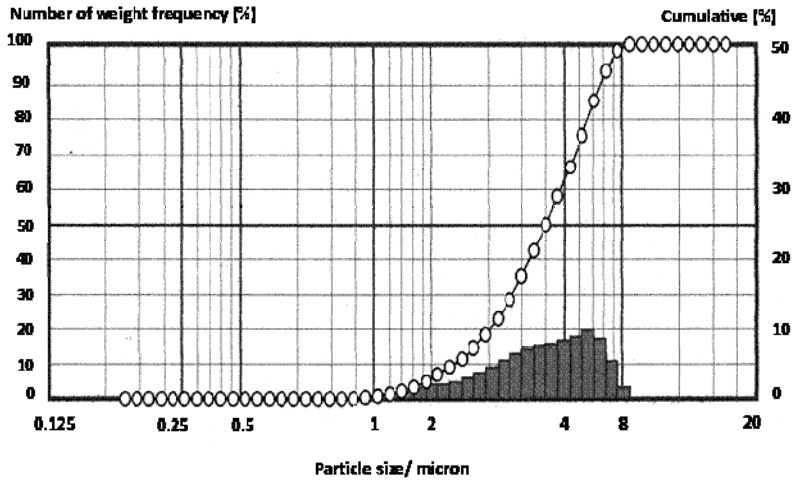

Figure 4. Average diameter distribution of the ganoderma particles at 200 °C.

Conclusion

The supercritical fluids, instead, can produce new and improved products with new and advanced processes. Manufacture of fine particles is one of the major supercritical fluids (SCF) applications. The SCF technology has been employed to form fine particles of pharmaceuticals and chemicals for many years. In order to combine the extraction technique with particle formation process, an original system was designed. This system has a novel property of being a combination of two different chemical operation systems. It makes us save energy, time and money with products being better in quality.

Apparatus was designed for experiments in the temperature range from 25 to 200 °C and pressures up to 10 MPa. The process was based on the solubility difference of the material in supercritical fluids at high and low pressures, respectively. Investigation of counter flow mechanism showed that there are some handicaps in formation of particles because of flow mechanism in the system design. So, the system was modified to concurrent flow type and nozzle was installed in order to spray the extract solution in air assistance. When the temperature was increased from 100 °C to 200 °C produced a large decrease of particle size and a narrowing of particle size distribution. At pre-expansion temperature of 200 °C very small particles with mean particle size about 4 μm were obtained. The molecular weight values of extracted ganoderma triterpenoids were distributed around 500 ~ 1000 g/gmol. To conclude, a novel design of a new system was succeed including extraction-air assisted particle formation processes in order to combine sub-critical water extraction system together with spray drying technique for energy and time, following them monetary saving.

Acknowledgements

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Effects of clarification, pasteurization and concentration on the tannins and antioxidant activity of pomegranate juice

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This study was conducted to determine the changes in high molecular weight polyphenols (tannnis), total polyphenols and antioxidant activity of pomegranate juice produced either from arils or whole fruits. Tannins are classified as hydrolyzable tannins (polymers of gallic or ellagic acid) and condensed tannins (polymers of catechins and epicatechins). Tannins were purified with Sephadex LH-20 gel chromatography and then analyzed by HPLC. The juice obtained from whole fruits and arils had 970 and 160 mg GA equivalent/L tannins, respectively. These results clearly show that most of the tannins were located in the rinds of pomegranates. Similarly, the juice obtained from whole fruits had also 34% more polyphenols, 54% more condensable polyphenols and 22% more antioxidant activity. Clarification decreased the tannins by 73%, total polyphenols by 23–38%, condensable polyphenols by 12–53% and antioxidant activity by 32–36%. On the other hand, pasteurization caused the increase in tannins by 46% and total polyphenols by 17%, but caused the decrease in condensable polyphenols by 29% and antioxidant activity by 7%. The concentrates obtained from non-clarified pomegranate juices had 32% more polyphenols, 29% more tannins and 28% higher antioxidant activities than those obtained from clarified juices. The tannin content in pomegranate juice also closely correlates (r=0.969) with their antioxidant activity. These results demonstrated, for the higher antioxidant activity pomegranates should be pressed with their rinds. On the other hand, since the tannins cause turbidity and contribute to astringent taste, some removal of tannins is necessary for technological as well as consumption reasons.

Changes in anthocyanins and color of black mulberry juice during clarification and heating

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This study was conducted to study the effects of clarification and heating on anthocyanins and color of black mulberry juice. The juice samples were heated at 70°C for 9 h, 80°C for 7.5 h and 90°C for 5 h. The color changes during clarification and heating of black mulberry juice were evaluated with monomeric anthocyanin contents and polymeric color analyses. Monomeric anthocyanin content of juice samples was analyzed by the pH-differential method. Polimeric color was measured spectrophotometerically by sulfite-bleaching method. After pressing, the juice was first depectinized and then clarified with bentonite, gelatin and kiselsol. Depectinization and fining processes resulted in 2% and 13% losses in monomeric anthocyanin content of mulberry juices, respectively. Immediately after pressing, the polymeric color ratio of black mulberry juice was determined as 1.68% and this ratio remained unchanged after depectinization (1.72%). Fining process have caused an increase in polymeric color ratio (4.69%). Analysis of kinetic data suggested first-order model for monomeric anthocyanin degradation during heating of black mulberry juice. Half-live values for anthocyanin degradation were found to be 11.75, 6.27 and 3.36 days at 70°C, 80°C and 90°C, respectively. The formation of polymeric color during heating also fitted to first-order (or may be zero-order) reaction kinetics. Recation rate constants for formation of polymeric color were 0.1285, 0.1299 and 0.2697 days at 70°C, 80°C and 90°C, respectively. Comparing half-live periods and reaction rate constants, the degradation of anthocyanins and formation polymeric color during heating of mulberry juice was highly dependent on temperature.

Key words: Anthocyanins, clarification, color, black mulberry juice, concentrate, stability.

Flavonoid changes in industrially processed and stored onions

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Abstract

Flavonoids are bioactive compounds which are known for their potential healthful effects. While onion is a vegetable widely found and eaten all over the world, it is the richest and most commonly consumed source of dietary flavonoids. It is known that different processing and storage conditions cause different changes in flavonoid contents of fruits and vegetables.

The aim of this study is to evaluate the effects of different packaging atmospheres, storage periods, storage temperatures, and light condition on stability and contents of flavonoids in sliced raw and fried onions. Onions purchased from a vegetable market hall were subjected to two different industrial processing conditions including slicing and deep frying of sliced onions.

Sliced raw onions were packed under atmospheric, nitrogen and vacuum conditions, then these packed onions were stored at room temperature with light and dark conditions, at +5 °C and -18 °C with only dark conditions for 1 day, 1 week, 2 weeks and 3 weeks periods. Deep frying process for sliced onions was carried out by applying 30 second deep frying at 141 °C in rapeseed oil. Fried onions were stored at room temperature with light and dark conditions for 1 week in atmospheric, nitrogen and vacuum packages and for 3 weeks only in nitrogen and vacuum packages, while they were stored at +5 °C and -18 °C with only dark conditions for 1 week, 2 weeks and 3 weeks periods in atmospheric, nitrogen and vacuum packages. In order to identify and quantify flavonoids found in aglycon and glycoside forms in onions the flavonoids were extracted from freeze-dried samples by accelerated solvent extraction and subsequently determined by ultra performance chromatography (UPLC).

The total flavonol content of unprocessed raw onion sample is 1632±96 µg q.e./g sample (dmb) with 961±58 µg q.e./g d.w Q-3,4'-diglucoside content and 588±38 µg q.e./g sample (dmb) Q-4'-glucoside content. In general total flavonoid content decreased with increasing storage period at room temperature. Important decreases in main quercetin glucosides Q-3,4'-diglu. and Q-4'-glu. were detected with an increase in quercetin aglycone content at room temperature. Generally, there were no distinct changes in total flavonoid contents and no important increase in the content of quercetin aglycone at +5 °C and – 18 °C in nitrogen atmosphere and vacuum packaging conditions. Main glucosides and total flavonol content in fried onion samples were higher than fresh onions. Light condition had lower flavonol content than dark condition with a quercetin aglycone production both for raw sliced and fried onions at each storage condition usually. Quercetin aglycone production was much more in light condition than dark condition during storage period both for raw sliced and fried onions.

As a result of this study; deep-frying of onion slices has resulted with an increase in flavonol content. For preservation of the flavonoid content it is recommended to keep fresh onions at normal air conditions while storing at room temperature for short time, whereas at nitrogen and vacuum atmospheres for longer storage at +5 °C and – 18 °C. On the other hand, for fried onions, at all storing temperatures vacuum application is found to be the favorite atmospheric condition from the point of preserving flavonoid content.

Introduction

Flavonoids are divided into six main subclasses according to their structures: flavones, flavonols, flavanones, isoflavones, flavanols (including catechins), and anthocyanins (Lagiou et al., 2004; Spencer,

2006; Nemeth and Piskula, 2007). Onions are one of the main food sources of flavonols (Amarowicz et al., 2009). Quercetin mono- and diglucosides are major flavonols in the onions, at the same time kaempferol, isorhamnetin and myricetin might also be available in different amounts (Søltoft et al., 2009). Quercetin-4'-*O*-monoglucoside and quercetin- 3,4'-*O*-diglucoside comprise 80% of the total flavonoids (Rohn et al., 2007).

Bioactive onion ingredients have anticarcinogenic, anticholesterol, antidepressant, antidiabetic, antifungal, anti-inflammatory, antimicrobial, antiosteoporosis, antioxidative, hypotensive, and antispasmodic functions (Lee et al., 2008). The type of processing used affects the health-promoting capacity and nutritional quality of plant products (Pérez-Gregorio, et al., 2011).

Pre-processing steps such as peeling, trimming, and chopping cause the greatest loss of flavonoids in onion (Ewald et al, 1999). Also, Kyle and Duthie (2006) found that domestic processing tecniques of chopping, shredding, peeling, and cooking changes flavonoid content by causing 21 to 54% losses of flavonols in onions besides formation of monoglucosides and free quercetin from diglucosides through glucosidase enzyme.

There are limited numbers of studies looking into flavonoid changes in sliced and deep-fried onions prepared by imitating industrial applications and stored at different storage conditions. For this reason, onions purchased from a vegetable market hall were subjected to two different industrial processing conditions including slicing and deep frying of sliced onions. Sliced raw onions were packed under atmospheric, nitrogen and vacuum conditions, then these packed onions were stored at room temperature with light and dark conditions, at +5 °C and -18 °C with only dark conditions for 1 day, 1 week, 2 weeks and 3 weeks periods. Deep frying process for sliced onions was carried out by applying 30 second deep frying at 141 °C in rapeseed oil. Fried onions were stored at room temperature with light and dark conditions for 1 week in atmospheric, nitrogen and vacuum packages and for 3 weeks only in nitrogen and vacuum packages, while they were stored at +5 °C and -18 °C with only dark conditions for 1 week, 2 weeks and 3 weeks periods in atmospheric, nitrogen and vacuum packages.

Materials and Methods

Chemicals and standards

The following chemicals were purchased: Methanol (MeOH, hypergrade for LC-MS and UPLC), formic acid (90-100%) and dimethyl sulfoxide (DMSO, >99 %) from Merck (Germany). Water for elution was prepared from distilled water using a Milli-Q system (18MΩ, Millipore, USA).

The UPLC grade standards; quercetin (Q) and isorhamnetin (I) were purchased from Extrasynthése (Genay Cedex, France), quercetin-3,4'-diglucoside (Q- 3,4'-diglu) was purchased from Polyphenols (France), quercetin-7,4'-diglucoside (Q-7,4'-diglu), rutin (Q-3-rutinoside) were purchased from Apin (Abingdon, United Kingdom), quercetin-4'-glucoside (Q-4'-glu) was purchased from Plantech (Berkshire, United Kingdom), quercetin-3- glucoside (Q-3-glu, purity >90%) was purchased from Sigma–Aldrich (Steinheim, Germany), kaempferol (K, purity 96%) was purchased from Fluka (USA).

Samples

The onion sample (*Allium cepa* var. *zittauer*, 60-80 mm) used for analysis was purchased from a wholesale vegetable market hall. Onions were conventionally grown at Lolland, Denmark, in 2010.

Sample preparation

For storage condition experiments of raw sliced onions, forty eight onions were used as six batches. For each batch, different parts of eight onions mixed well and shared equally between eight trays that are available for eight different treatments. The top and bottom parts of onions were removed and outer brown skins were peeled. Then onions were placed in the slicing machine (Robot Coupe R502; knife Robot

Coupe E/S5, France) in twain and sliced. Trays with sliced onions were put in PA/PE bags and packed in nitrogen (25 mbar evacuation, 300 mbar nitrogen), air and vacuum atmospheres by using the packaging machine (Multivac, Germany).

Samples for light condition were placed in a cabinet including four fluorescent lamps (Radium, Spectralux® Intra NL-T8 18W/827, Germany) while for dark condition black plastic covers were used. Remaining samples for dark condition of 5 °C and -18 °C were placed fridges until they complete their storage period.

Samples were freeze-dried (Merck Eurolab, Christ LMC-1, Germany) at 1×10^{-3} bar for 2-4 days and afterwards crushed and homogenized by a blender (Braun, Germany). The powdered samples were stored at -21°C with a nitrogen atmosphere in PA/PE bags until extraction.

In order to see frying process effect on flavonoid content of onions, the onions samples were subjected to frying at 141 °C, during 30 sec. in rapeseed oil. Before frying operation, onion samples were sliced with slicing machine, then all collected onions were mixed in a big pot, weighed as approximately 120 g sliced onions for each batch of frying. After that, weighed onions put in the metal basket then deep fried in a deep fryer unit (FKI, VSCF4, Denmark) including 8 liters of rape seed oil. Also, the oil was agitated to provide homogenous temperature distribution at 291 rpm by an agitator (Eurostar Digital, IKA Labortechnik, Germany). After frying operation the basket including fried onions was put on paper towels. Then onions were waited in metal cowls during 10 minutes for cooling to room temperature. After that, all fried onions were collected in a big transparent plastic bag (PA/PE) and kept in 5°C until the finish of operation. Then fried onions were mixed properly, weighed as portions for their storage conditions and packaged. Finally, all packaged onions were put their storage conditions.

Pressurized liquid extraction

The extractions were carried out using an ASE-350 (Dionex, Sunnyvale, USA).To perform an extraction, firstly glass fiber filter (d=27 mm, Dionex) was placed to the bottom of the 5 mL size cell (32 mm) and bottom end cap were tightened. Then 0,5 g freeze dried and powdered sample mixed with Ottowa sand (particle size 20–30 mesh, Fisher Chemicals) was loaded into a sample cell, and the cell was filled with Ottowa sand to edge of the loading hole and the top cap was tightened by hand onto the cells. After that the prepared sample cells were loaded onto a cell tray and collection vessels (bottles or vials) were loaded into a collection tray. The extraction parameters were: temperature: 40 °C, pressure: 1500 psi, static extraction time: 5 min, heating: 5min, static cycles: 2, flush volume: 20% of cell volume, purge time with N_2: 60 s. After extraction approximately 10 ml extracts were obtained and the obtained extracts were diluted in 20 mL glass flask by 65% methanol in water solvent and kept at -20 °C until UPLC analysis.

Identification of flavonoids by UPLC

The UPLC system consists of an AcQuityTM UPLC equipped with a binary pump system Waters (Milford, MA, USA) using an AcQuity UPLC HSS C18 column (1.8 µm, 100 mm × 2.1 mm i.d.). During the analysis, the column was kept at 30°C and the flow-rate was 0,46 mL/min. The mobile phase was eluent A, MilliQ water/methanol (90/10, v/v) with formic acid from 0,01 % of water, and eluent B, methanol (100%). The elution started with 5% of eluent B, and from 0,09 min, then eluent B was linearly increased to 50 % until 5,6 min. After that, eluent B was increased to 60% in 2,4 min and then stayed isocratic for 1 min. Then the gradient was turned back to initial conditions in 1 min, and the re-equilibration time was 5 min giving the total run time 15 min. The injection volume was 2.5 µL, and all dilutions of the sample extracts were filtered through 0.22 µm filter (Sartorius Minisart) before the chromatographic analyses.

The UPLC was coupled to a Photodiode Array (PDA) detector AcQuity UPLCTM (Waters, Milford, MA, USA). The wavelengths in the PDA detector were set at 210- 450 nm, resolution was 1,2 nm and sampling rate was 1 points/sec. The software used was EMPOWER for instrument control and data acquisition.

Statistical analysis

Results are the means ± standard deviations from duplicate extracts of each sample. Statistical analyses were done by using one-way analysis of variance in SPSS.19.The means were separated by Duncan's multiple range test in groups coded by a,b,c, and d if significant differences were detected at the 0.05 level.

Results and Discussion

Identification was carried out by comparison of the retention times and UV spectras of the peaks obtained from UPLC measurement of onion samples and commercial standards. At the same time quantification was completed by comparing peak areas of known amounts of quercetin standard with the peak areas of the onion flavonoids, and expressed as quercetin equivalents in dry weight of the sample. Commercial standards of the quercetin 3,7,4'-triglucoside, isorhamnetin 3,4'-diglucoside, isorhamnetin 4'-glucoside weren't used for UPLC analysis. However, obtained peaks of these flavonoids in samples compared with literature in terms of their retention times and PDA images.

The total flavonol content of unprocessed raw onion sample is 1632±96 µg q.e./g d.w with 961±58 µg q.e./g d.w Q-3,4'-diglu. content and 588±38 µg q.e./g d.w Q-4'-glu. content. Total flavonol content can be expressed as 211±12 µg q.e./g f.w also.

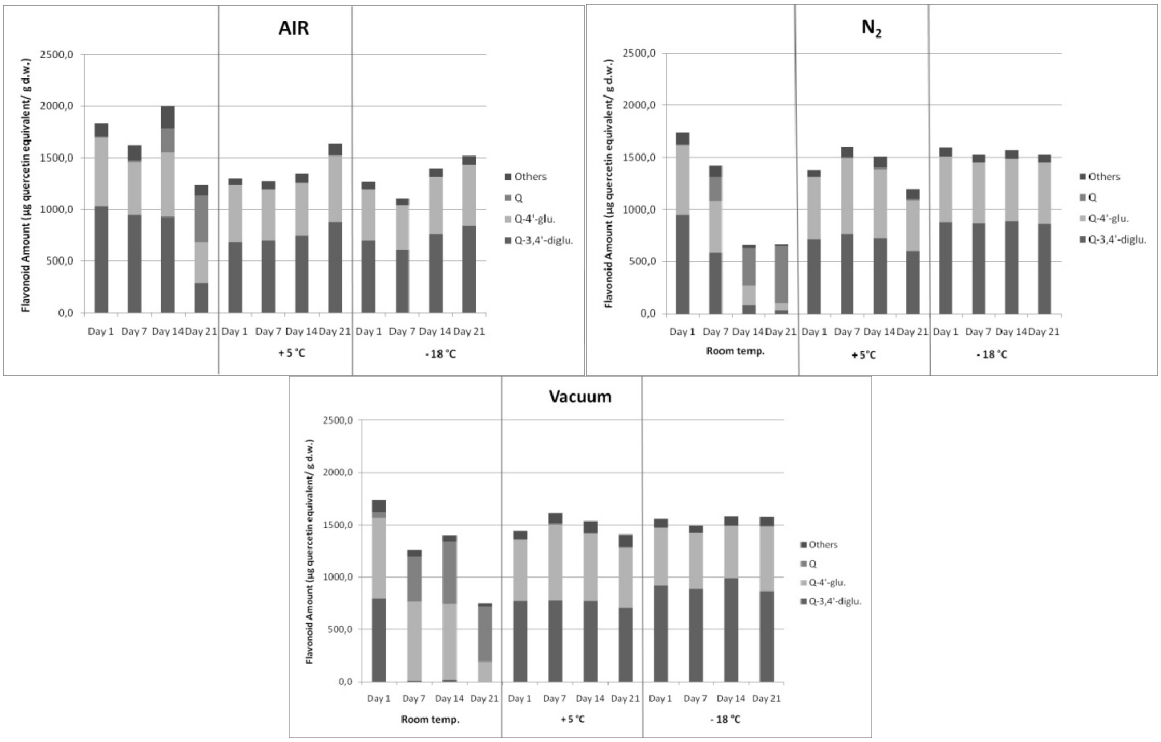

Figure 1. Comparison of the effects of different storage temperatures on the levels of the main flavonols in raw sliced onions for three different storage atmospheres under darkness.

Others Σ (quercetin 3,7,4'-triglucoside, quercetin 7,4'-diglucoside, isorhamnetin 3,4'-diglucoside, quercetin 3-glucoside, isorhamnetin 4'-glucoside, kaempferol, and isorhamnetin). Significant differences between total amounts were separated by Duncan's multiple range test in groups coded by a and b.

From the graphics for all packaging atmospheres it is seen that at room temperature total flavonoid content decreased with increasing storage period. Marked decreases in main quercetin glucosides Q-3,4'-diglu. and Q-4'-glu. were observed while increase in quercetin aglycone content occurred. This increase might

be due to combined effect of room temperature and storage period, or glucosidase activity at room temperature during storage period. Processing temperature has different effects on deglycosylation of flavonoids. Boiling of onion didn't cause deglycosylation but quercetin transferred to the boiling water (Nemeth et al., 2004); while roasting of onion at 180 °C led to both degradation and change of quercetins to other compounds (Lee et al., 2008). In macerated tissues of onion, a rapid degradation of Q-3,4'-diglu. to quercetin monoglycoside and aglycone due to activity of onion flavonol glucosidases was observed in previous studies (Nemeth et al., 2003). It was also found that water condensation occurring on the inner walls of packaging material containing sliced onion samples caused increase in quercetin aglycone and Q-4'-glu. content with a decrease in Q-3,4'-diglu. content (Perez-Grégorio et al., 2011). In our study water condensation was also observed on the inner walls of transparent plastic bags during storage period at room temperature conditions.

Day 1 values of room temperature at all packaging atmosphere conditions were higher than all values of other storage temperatures. There were no distinct changes in total flavonoid contents and no important increase in the content of quercetin aglycone at +5 °C and – 18 °C in nitrogen atmosphere and vacuum packaging conditions. In a previous study about packaging of chopped onions it is stated that packaging under vacuum or in low oxygen permeability modified atmosphere packaging had no effect on quercetin content (Perez-Grégorio et al., 2011).

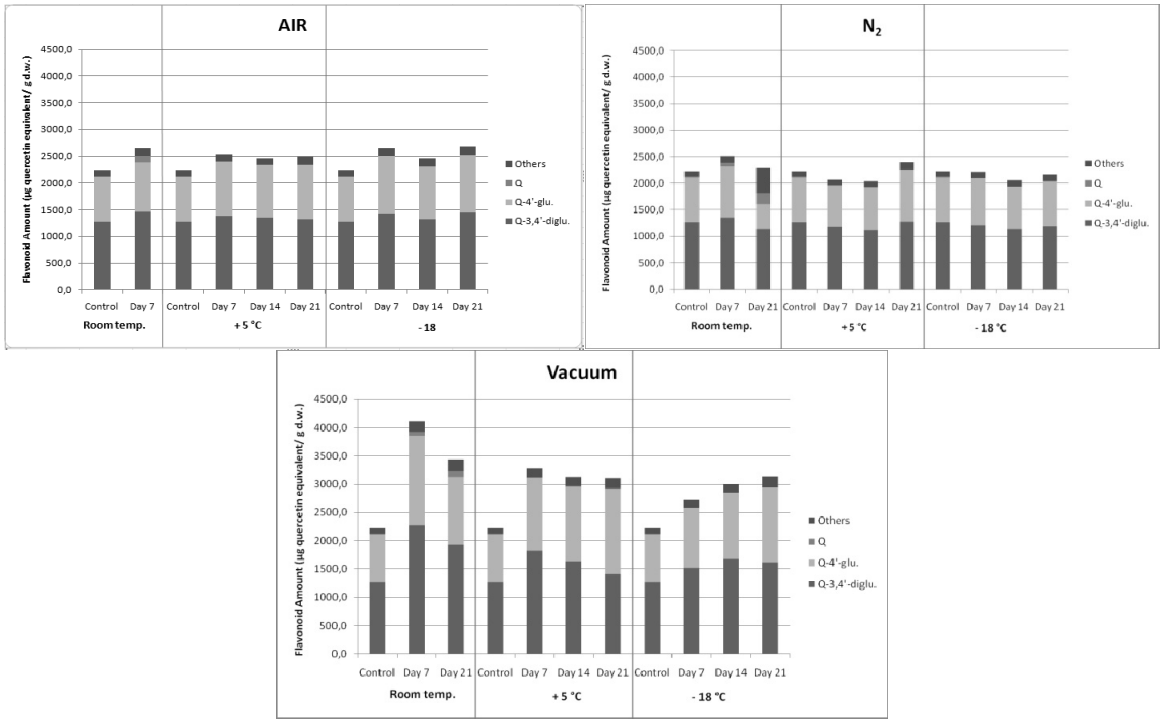

Figure 2. Comparison of the effects of different storage temperatures on the levels of the main flavonols in fried onions for three different storage atmospheres under darkness.

Others Σ (Q-3,7,4'-triglu., Q- 7,4'-diglu., I-3,4'-diglu., Q-3-glu., I-4'-glu., K, and I). Significant differences between total amounts were separated by Duncan's multiple range test in groups coded by a and b.

The amounts of main glucosides and total flavonol content in fried onion samples were higher than fresh onions. According to literature main quercetin glucosides found in onion are reasonably heat stable (Wach et al., 2007, Nemeth et al, 2003); so, a deep frying process including high temperature and short time parameters might cause an increase in flavonol amounts. Some domestic cooking methods like baking and sautéing causes increases in quercetin content between 7-25% (Amarowicz et al., 2009). During technological processes the amount of flavonoids may increase by releasing from food matrix (Nemeth et

al, 2003). In addition, cooking and boiling caused more losses in quercetin content than frying because both flavonoid breakdown during cooking and conjugated quercetin extraction from onion samples by hot water was higher than hot sunflower oil; also no free or conjugated flavonoids found in oil after frying (Crozier et al., 1997). Therefore, flavonol losses from onion samples to the oil couldn't be expected during our deep frying experiment. In another study, some green vegetables were subjected to boiling, steaming and microwaving processes, and total phenolic content of pepper, broccoli and green beans was significantly increased after these cooking methods (Turkmen et al., 2005). However, potato samples deep-fried at 191°C for 1 min. in canola oil had increased levels of chlorogenic acid, caffeic acid, (-)epicatechin, p-coumaric acid and vanillic acid but decreased quercetin dihydrate while compared to uncooked samples (Blessington et al., 2010).

The quercetin aglycone amounts in nitrogen atmosphere and vacuum packaged fried samples after three week storage period at room temperature were found lower than that of raw sliced samples probably due to inactivation of glucosidase activity after deep frying operation. Through the storage period there weren't any marked increase or decrease in total flavonol content in the samples of air and nitrogen atmosphere packaged samples. Awad and Jager (2003) reported that during air storage at low temperature and in dark conditions, biosynthesis of anthocyanins and flavonoids in blueberry, pomagranates and strawberry might increase. In strawberry ellagic acid, catechin, quercetin, and kaempferol derivatives increased during storage period but not due to CO_2 concentration in the storage atmosphere. Also, flavonoid and hydroxycinnamic acid derivatives in nectarine, cherry, grape and strawberry fruits stayed constant during air storage.

Light condition had lower flavonol content than dark condition both for raw sliced and fried onions for each storage condition. Quercetin aglycone production was much more in light condition than dark condition during storage period both for raw sliced and fried onions.

Conclusion

It is important to keep nutritional properties of foods during processing. In this study deep-frying of onion slices has resulted with an increase in flavonol content. Because it is not possible to consume sliced and fried onions stored at room temperature and +5°C after a long storage period, processed onions kept at –18°C can be consumed by preserving flavonol content at the same time.

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Variation of textural properties in pears dried by different solar methodologies

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The food textural properties, together with the appearance and flavor, determine the quality of food products, and therefore, their acceptance by consumers. Hence it is important to control and predict the changes that occur in the texture of foods with processing operations, such as drying. In Portugal, pears are subject to an artisan process of drying in direct open-air sun exposure. However, the drying process does not provide the current standards of quality and, therefore, recent investigations have emerged with alternatives to traditional drying processes. The objectives of this study were to determine the changes that occur in texture with the drying process, and compare the texture of pears dried by two different drying processes, namely the traditional and the drying in a solar stove with ventilation. Pears of the Portuguese variety *S. bartolomeu* were obtained from a local producer, both fresh and after traditional drying. Some of the fresh pears were then dried by the alternative method. The determination of the textural properties was done with a Texture Analyzer TA.XT.Plus from Stable Microsystems using the Texture Profile Analysis (TPA) method. From the results obtained, it was possible to observe that both drying process affected the texture. However, no important differences were seen when the two drying methods were compared with each other. These results allowed concluding that the alternative drying methodology can be used to replace the traditional one, without altering the properties of the final product, with respect to texture.

Nutritionally enhanced wheat bread products: mechanisms of beneficial action of ascorbic acid and chitosan dietary fibers

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Abstract

DSC studies show that all water in bread is bound to bread components (no free unbound water – temperatures of melting and crystallization peaks considerably differ from melting and crystallization temperatures of free bulk water). Decrease of loosely bound freezable water content was found during staling. More rapid decrease of loosely bound freezable water content was observed in the presence of chitosan. The enthalpy of water crystallization in bread crumb considerably decreases if ascorbic acid was added to bread. Two water crystallization exothermic peaks (at temperatures 233 K and 263 K) were observed in fresh bread crumb containing 3% chitosan + 0.6 % ascorbic acid. After 2 and 7 days storage crystallization exothermic peak at temperatures 233 K disappeared and only one crystallization peak at 263 K was observed. Additional exothermic peak at low temperature 233 K can be attributed to strongly bound freezable water. The reasons for the increase of free lipids in fresh bread containing chitosan oligosaccharides and a decrease in the absorption of dietary lipids from the small intestine are to a great extent similar. DSC studies of water behaviour in bread allow to propose a mechanism for ascorbic acid and chitosan action.

Introduction

Recent years have seen increased interest of consumers, researchers, and the food industry into how food products can help maintain the health of an individual. Dietary fibres could be used as functional ingredients because they provide numerous health benefits that may include not only digestive health, but also cardiovascular health, and general wellness. But adverse effects of flour replacement by dietary fibers in bread making (disruption of the starch-gluten matrix in dough, reduction in starch availability for gelatinization, decreased loaf volume, lowered gas retention) have been observed. Due to the existence of amino groups, chitosan possesses positive charge, so it can bind negatively charged substrates such as lipids and bile acids, as well as adsorb on starch granules and protein surfaces in bread. It is well known that properties of confined water differ from the properties of free three dimensional bulk water. Water in various polymer and biopolymer systems, including food products, can be subdivided into free water (bulk water), which freezes at the usual freezing point and is not influenced by the biopolymer surfaces, the unfrozen bound water (tightly bound water), which does not freeze and intermediate water (loosely bound water), which freezes below the usual freezing point. The loosely bound water is connected to the hydrophilic polymer groups and melts at a lower temperature due to its interaction with the polymer (1). The main objective of this study was from the changes of the state and content of water to identify molecular mechanisms through which chitosan and ascorbic acid influence bread structure and staling.

Materials and methods

Dough was prepared based on the following formulation: 500 g wheat flour, 35 g sugar, 17 g dry yeast, 8 g salt, 10 g butter, chitosan 10 g, ascorbic acid 2 g and 260 g water.

Chitosan with viscosity 29 cP and degree of deacetylation 97% was kindly supplied by Primex (Siglufjordur, Iceland). Dough mixer "MONO" (Swansea, UK) was used to make dough at 2 stirring rates during 10 minutes. The dough was allowed to proof for 25-30 minutes at 38 ^{0}C and relative humidity 72% and cut to pieces with weight 65 g. The rolls were baked in confectionery oven "MONO" (Swansea, UK)

at 200 ^{0}C for 12 minutes. The rolls were then cooled at room temperature for 30 minutes and packed in polypropylene bags.

Differential scanning calorimetry (DSC) thermograms were obtained using a differential scanning calorimeter DSC "Mettler 300" (Mettler-Toledo AG, Schwerzenbach, Switzerland*)*. Approximately 10 mg bread samples were placed into aluminum pans, and then a lid was secured by crimping. The samples were placed in the sample compartment at T = 25 °C and cooled to T = -50 °C at 10°C/min and held at T = -50 °C for 5 min. Samples were then scanned from T = -50 °C to T = 25 °C with a heating rate of 5°C/min. Each measurement was performed in triplicate.

Results and discussion

Chitosan can affect the migration and distribution of water and lipids in bread crumb and crust during storage. DSC studies show that all water in bread is bound to bread components (no free unbound water – temperatures of melting and crystallization considerably differ from melting and crystallization temperatures of free bulk water). The exothermic peak of water crystallization in bread crumb was observed at 249 K, Figure 1a (the crystallization temperature of free bulk water is 273 K). So this water is loosely bound to polymer molecules. The crystallization enthalpy of loosely bound water in bread crumb decreased essentially after 2 days of bread storage and the peak completely disappeared after 7 days of bread storage at room temperature, Figure 1a. The exothermic peak after 2 days of bread storage shifted to 240 K. Bushuk and Winkler (2) reported that water is bound more to starch than to gluten, based on its higher water sorption capacity. In the crumb containing 3% chitosan crystallization peak was observed at 244 K and completely disappeared already after 2 days storage, Fig.1b. It was suggested that the adsorption of polycationic chitosan onto the starch granule surface and the complex formation between starch and chitosan, prevented starch from taking up water released from gluten during bread aging (3). This water migrates to the crust of the bread. Decrease of water crystallization enthalpy (decrease of the content of loosely bound water) in bread crust with storage time was observed for control bread, Figure 2a. But in bread containing 3% chitosan water crystallization enthalpy in bread crust has maximum value after 2 days storage, Figure 2b. It means that content of loosely bound water in the bread crust increases during first 2 days of storage for bread containing chitosan and then decreases in the time interval from day 2 to day 7. The similar behavior was observed for bread crust containing 3% chitosan + 0.6 % ascorbic acid, Figure 2c. So only chitosan affects water behavior in bread crust and not ascorbic ascorbic acid.

Water in fresh bread crumb containing 3% chitosan + 0.6 % ascorbic acid has 2 crystallization exothermic peaks (at temperatures 233 K and 263 K), Fig 1c. Crystallization enthalpy is lower than crystallization enthalpies of control bread and bread containing 3% chitosan and decreases over storage time. After 2 and 7 days storage crystallization exothermic peak at temperatures 233 K disappeared and only one crystallization peak at 263 K can be indicated. Additional exothermic peak at low temperature 233 K can be attributed to strongly bound freezable water. Taking into consideration that melting point depression and crystallization point depression of liquids confined in a porous material is inversely proportional to pores size we can suppose that additional pores with lower size were formed in bread crumb when ascorbic acid was added. During storage the interaction of water with starch and gluten macromolecules increases, partly due to further decrease of pore sizes, and strongly bound freezable water converts into non freezable tightly bound water. The sulfhydryl-disulfide interchange reaction was used to explain the action of ascorbic acid. The network of disulfide bonds formed in the gluten structure plays a significant role in ***retaining*** the ***carbon dioxide produced*** during fermentation and results in higher volume and improved texture (4). The formation of additional pores with lower size in bread crumb when ascorbic acid was added can be related to the formation of the network of disulfide bonds in the gluten structure.

It can be suggested that chitosan was bound to outside part of the starch granules and loosely to the gluten. Increase of free lipids in bread containing chitosan can be explained by blocking the surface of gluten or the outside part of the starch granules (5). The reasons for the increase of free lipids in fresh bread

containing chitosan oligosaccharides and a decrease in the absorption of dietary lipids (triacylglycerol and cholesterol) from the small intestine are to a great extent similar. Chitosan has good bioadhesive characteristics to the mucosa of small intestine and regulates the absorption and transport of different dietary lipids by the human small intestine and similarly chitosan regulates the absorption and transport of lipids in bread crumb.

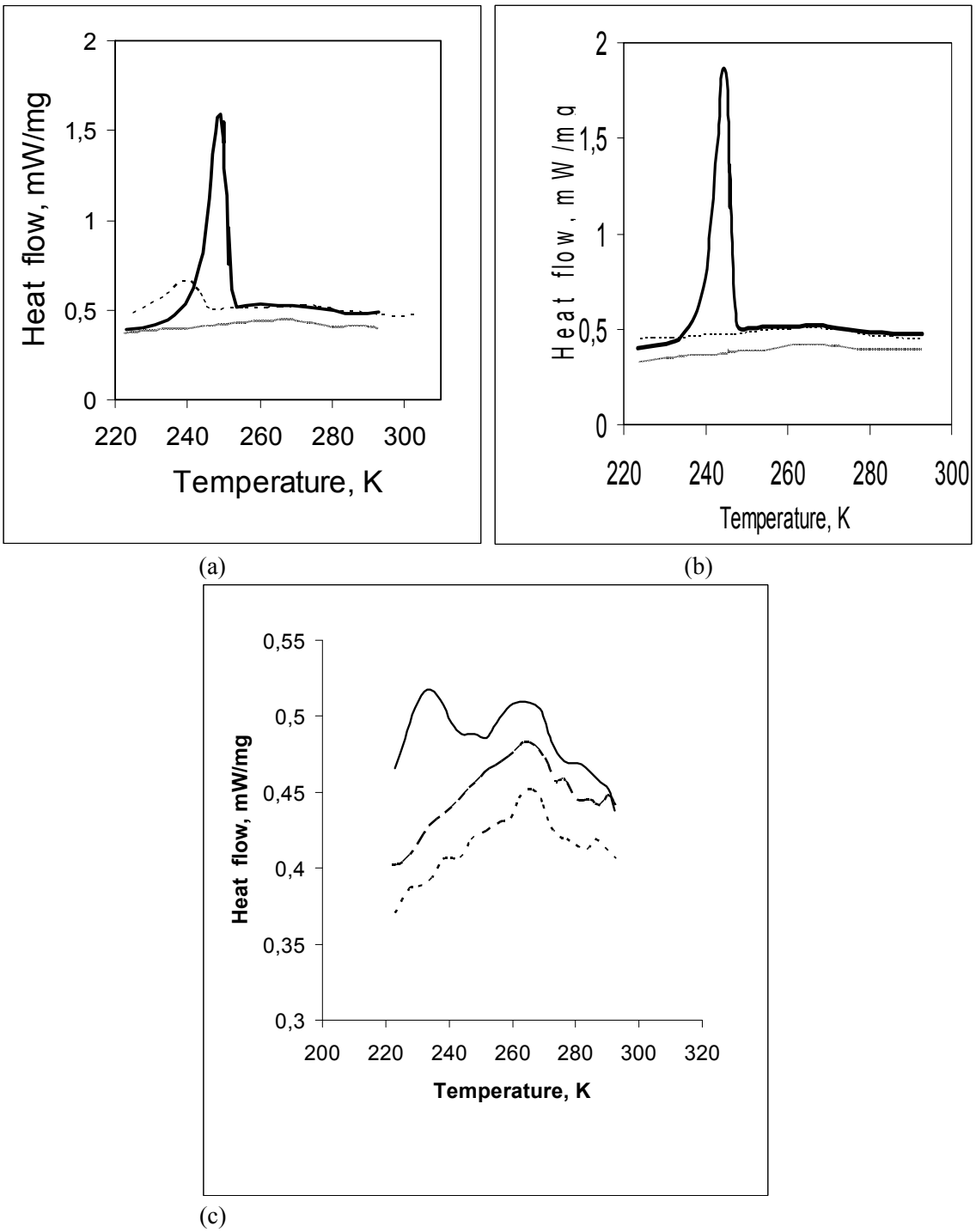

Figure1. Crystallization thermograms of water in bread crumb (a) in bread crumb containing 3% chitosan (b) and in bread crumb containing 3% chitosan + ascorbic acid (c) after 0 (solid line), 2 (dashed line) and 7 (dotted line) days of storage.

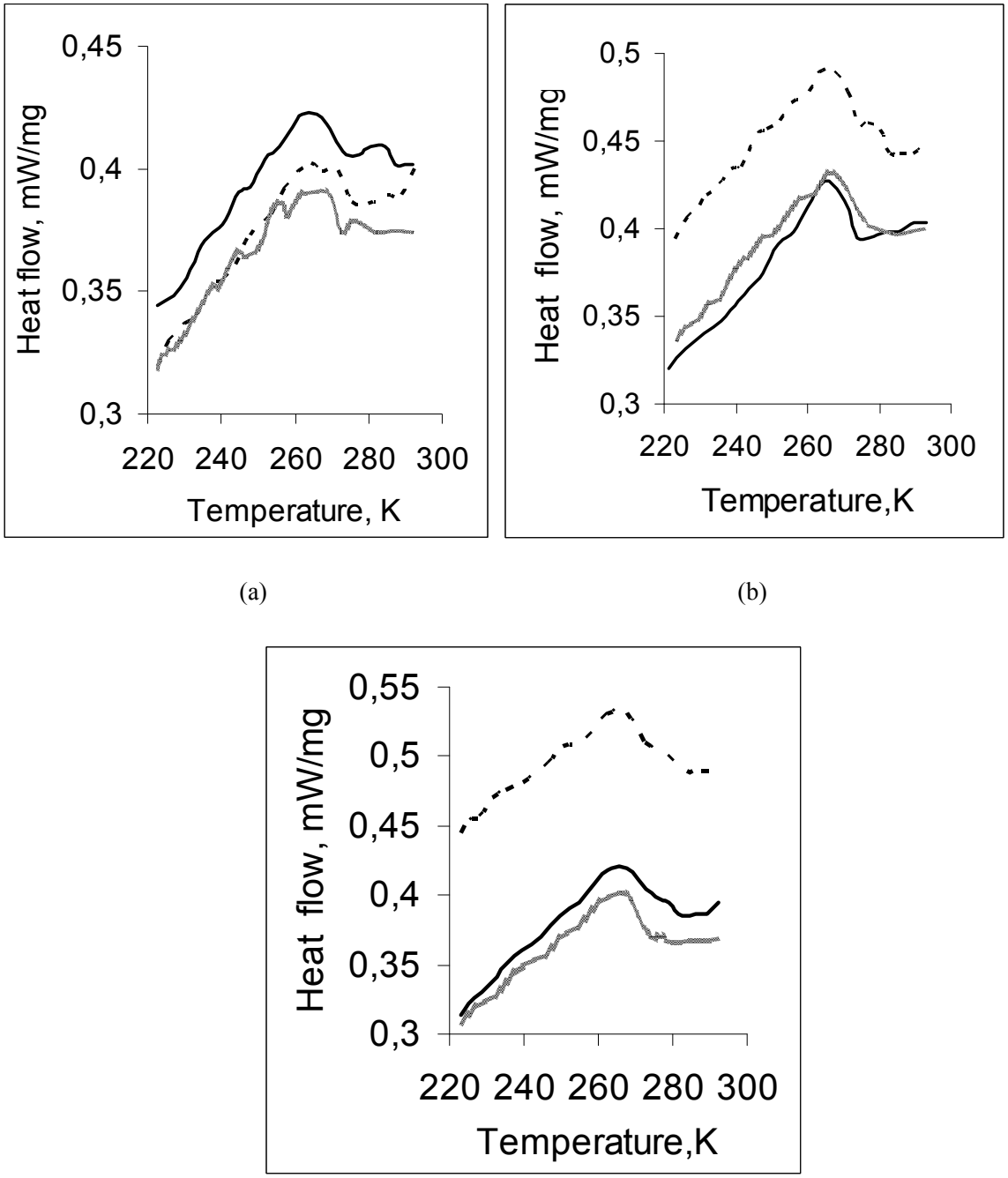

Figure 2. Crystallization thermograms of water in bread crust (a) in bread crust containing 3% chitosan (b) and in bread crust containing 3% chitosan + ascorbic acid (c) after 0 (solid line), 2 (dashed line) and 7 (dotted line) days of storage.

Conclusions

Chitosan and ascorbic acid affect the content and distribution of loosely bound water in bread crumb and crust and in such a way regulate a bread staling rate and potentially can affect health beneficial properties of bakery products. Chitosan binding to outside part of the starch granules and gluten surface affects bread staling rate through the changes in water distribution between crumb and crust and between starch and gluten and water migration rate. Ascorbic acid affects the network of disulfide bonds formed in the gluten.

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Acceptability of a chickpea based gluten-free bread and rusk by celiac and non-celiac persons

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Abstract

Acceptability of a bread and rusk made of branny chickpea flour as the main ingredient was assessed by celiac and non-celiac persons. The flour of the products was composed of branny chickpea flour and commercial gluten-free flour in the ratio of 100:0, 80:20, and 60:40. The acceptability of bread slices and rusks were tested by untrained 35 celiac and 33 non-celiac assessors using a nine-point hedonic scale. In the hedonic scale, the scores of 1, 5, and 9 corresponded to "dislike extremely," "neither like nor dislike," and "like extremely," respectively. The results were evaluated using one-way ANOVA with α=0.05, in case of significant difference Tukey multiple comparison test was exploited. The samples were scored between 5.8 and 7.4 on average indicating that both products were accepted by both celiac and non-celiac assessors.

Introduction

Bread is a staple food in human nutrition for centuries. Turkish population provides a considerable portion of daily food intake from bread. Its daily consumption is reported between 300-500 g/person, and also it provides 45 % of energy and 47 % of protein requirement of the population (Talay, 1997).

Consumption of bread and other food products made of wheat, rye, and barley can cause various diseases in some individuals who are sensitive to gluten. Celiac disease, one of these, is an autoimmune disorder of the small intestine because of gluten sensitivity that can occur in genetically predisposed people of all ages (Stepniak and Koning, 2006). A strict lifelong gluten-free diet is the only known current treatment for celiac disease (Korus, 2009).

Different standards are adopted for accepting a manufactured food as gluten-free based on the gluten content. In the USA and Canada, the use of grains naturally containing gluten is not allowed in the production of gluten-free foods. In the United Kingdom, the use of wheat starch is allowed in the production of gluten-free foods (Gallagher et al., 2004). European Community classifies foodstuffs containing 20 mg gluten/kg as "gluten-free" and 20-100 mg gluten/kg as "very low gluten" (Anonymous, 2008). In Turkey, gluten-free foods are classified into two groups as "gluten-free" and "gluten-reduced" and the maximum limit of gluten is 20 mg/kg for the former and 200 mg/kg for the latter on dry basis (Anonymous, 2003).

Significant number of works is available particularly on gluten-free breads using various gluten-free ingredients (Gallagher et al., 2004). Corn and rice are common crops tested for the possibility for making gluten-free breads (Sabanis et al., 2009). Chickpea is a gluten-free crop and known to have many nutritional and health benefits (Zia-Ul-Haq et al., 2007). A reported work on its usability for the production of gluten-free bread is not reached in the literature.

The purpose of the present study which is a part of a whole project is to determine the acceptability of a chickpea based gluten-free bread and rusk in case such a product is manufactured for celiac patients. For this purpose chickpea flour added by commercial gluten-free flour or not was processed into bread and rusk and their acceptability was sensorially evaluated by celiac and non-celiac assessors.

Materials and Methods

Raw materials

Kabuli type chickpea was donated by a legume processor (Memişler Tad Bakliyat, Mersin, Turkey). Commercial gluten-free flour consisting of rice flour, maize starch, pectin and xanthan gum (Glutensiz Un, Sinangil, Konya, Turkey) was obtained from a local market together with other ingredients (yeast, sunflower oil, table salt and table sugar).

Batter, bread and rusk making

Batter making

Split or whole chickpea was grounded using a hammer type mill (Plant Mill, Şimşek Labor, Ankara, Turkey) with a 1 mm circular screen. The grounded chickpea was sieved through a screen (Retsch, Haan, Germany) of 250 μm and permeate was exploited in the flour formulation of the bread and the rusk. The flour of the samples consisted of the branny chickpea flour/gluten-free flour in the ratio of 100:0, 80:20, and 60:40. For every 100 g of the flour 110 g tap water, 6 g table sugar, 5 g sunflower oil, 3 g yeast and 1.6 g table salt were used. The flour and salt was mixed in yeast and sugar previously dissolved in water for 90 s with a mixer at level of 5 (Kumtel, Kayseri, Turkey). Sunflower oil was added into the mix and the batter was stirred further 30 s. The batter was poured into aluminum baking cups with dimensions of 110×110×42 mm in portions of 120 g. Cups were kept at 30 ^{o}C for 45 min for proofing the batter.

Bread making

Some cups from the batter preparation were kept at 175 ^{o}C for 50 min in a laboratory oven (ULP600, Memmert, Nürnberg, Germany) to obtain chickpea breads. The breads were removed from the cups and kept at ambient temperature for 2 h for cooling.

Rusk making

The remaining cups were kept at 175 ^{o}C for 40 min in the same oven. The cooked batters were removed from the cups, kept at ambient temperature for 2 h for cooling, sliced into 8 mm thickness using a slicer (Bench Top, Mataş, Bursa, Turkey), and the slices were toasted at 130 ^{o}C for 90 min to obtain chickpea rusks and kept at ambient temperature for 2 h for cooling.

Sensory evaluation

Sensory analyses of the breads and rusks were carried out by untrained 35 celiac and 33 non-celiac assessors. A nine-point hedonic scale was used to evaluate the overall acceptability of the samples. The assessors scored the samples on a scale of 1 to 9 (1=dislike extremely, 5=neither dislike nor like, 9=like extremely). Analysis of variance and Tukey multiple tests (α=0.05) were utilized using a statistical software (SPSS 11.5 for Windows 7.0, IBM, Armong, NY, USA) for analyzing the sensory data.

Results and Discussion

Celiac assessors graded the chickpea breads between 6.6 and 7.2, and the chickpea rusks between 6.5 and 7.4 on average (Figure 1). Non-celiac assessors pointed the breads between 6.1 and 6.7, and the rusks between 5.8 and 6.3 on average (Figure 1). In the nine-point hedonic scale, a grade greater than 5 for the tested product points that it is “liked” by assessors. Grades around 6 and 7 in the hedonic scale correspond to “liked slightly” and “liked moderately” by the assessors, respectively. Accordingly, all celiac and non-celiac assessors liked the products tested. The degree of the preference varied between celiac and non-celiac assessors and the former consistently “liked” the same products more than the latter (Figure 1).

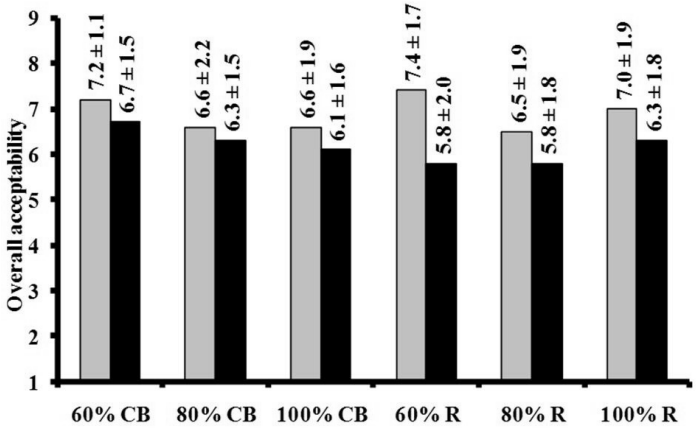

Figure 1. Acceptability of chickpea breads and rusks by celiac and non-celiac assessors. CB: Chickpea bread; R: Rusk, ■: Score of celiac assessors, ■: Score of non-celiac assessors (The first figure above the bars is for the average grade and the second after "±" sign is for the standard deviation of the grades)

The "liked" scores of the celiac assessors could be a "real liking" due to physiological reasons or an "artifact liking" due to psychological reasons, or both. The availability of suitable manufactured foods for celiac patients in Turkish market is extremely limited, and the existent ones are quite expensive compared to their gluten containing counterparts (Savran and Turhan, 2011). The new taste, the new chance for a cheap food and the limited availability of the gluten-free foods may have forced the celiac assessors to psychologically like the samples tested. The non-celiac assessors are expected to be free from such forcing effects, therefore their "liked" scores can readily be perceived as "real liking." Taking the "real liking" of the non-celiac assessors as the base line, the "real liking" is effective on the "liked" scores of celiac assessors. The consistently greater scores of celiac assessors than those of non-celiac assessors (Figure 1) for the same samples can be attributed to influence of the factors forcing toward the "psychological liking" to some extent.

Conclusions

The bread and rusk made of branny chickpea flour and the commercial gluten-free flour within the ratio of 100:0 and 60:40 is readily acceptable by celiac and non-celiac persons. Consuming breads and rusks from chickpea can provide new nutritious choices for both celiac and non-celiac population.

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The survey of health effects and the methods of reduction of acrylamide in heat-treated starchy foods

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Acrylamide (2-propenamide) is a low molecular weight hydrophilic compound known mostly for its use as a monomer in the production of polyacrylamide. Announcement made in 2002 by the Swedish National Food Administration reported extremely high levels of acrylamide in products that are consumed on a regular basis in rather large quantities such as potato crisps, roast potatoes, breakfast cereals and bakery products. Acrylamide is formed during cooking, frying and baking at temperatures exceeding 120°C of fatty, carbohydrate and asparagines-rich foods either as product of asparagines-glucose reaction (Millard reaction) or decomposition of triglycerides. Health effects and toxicological studies, carcinogen probable effects on human and problems of inhaling and consumption of acrylamide contained products and ways to minimize its presence in heat-treated starch-riched foods have been discussed. Some factors in reduction of acrylamide are varieties, harvesting year, fertilizer amounts, extraction rate and optimization of production technology. The latter strategy not only comprises technological measures such as temperature and time control. The research showed acrylamide in pilot plant experiments was reduced with increasing fermentation time. Also product formulations and the use of additives and the effect of various material such as divalent cations (Ca^{+2}) that prevents acrylamide formation completely whereas monovalent cations (Na^{+}) almost halved the acrylamide formed in the model system are crucial. NaCl plays an ambiguous role: whereas low doses up to 2% lowered acrylamide by inhibition of the enzyme activities, higher addition remarkably increased the contents due to growth inhibition of the yeast. The influence of addition of cysteine to the dough resulted in significantly lower acrylamide content. Furthermore, enzyme-bearing bakery improvers had no influence on acrylamide formation. Reduced baking temperature and prolonged heat treatment is favorable. Convection oven seem to enhance acrylamide formation compared to deck oven.

Nutritional assessment of functional beverage supplemented with barley β-glucan

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Abstract

Barley contains active ingredient, β-glucan which is a good source of soluble fiber. The barley flour was prepared from Pakistani barley variety (Haider-93) and analyzed for its chemical composition. The barley flour possessed 11.48% total dietary fibre and 4.87% β-glucan content. The β-glucan extracted from barley flour contained 75.05% soluble dietary fiber 10.25%, insoluble dietary fiber and 85.30% total dietary fiber. The beverage was prepared by incorporating β-glucan at 0, 0.2, 0.4, 0.6, 0.8 and 1.0% levels. The L*-value of beverage increased while b*-value decreased progressively by incorporation of barley β-glucan. The viscosity and acidity of the beverage also improved significantly with β-glucan level. Sensory evaluation revealed that the beverage containing 0.2 and 0.4% β-glucan showed similar response as that of control. Overall, the incorporation of β-glucan upto 0.8% did not significantly affect sensory parameters of flavor, color and acceptability.

Key words: Barley, β-glucan, functional beverage, sensory attributes

Introduction

Barley is a cereal belongs to the annual grass *Hordeum vulgare* and serves as a major animal feed crop, with minor proportions used for productions of healthy human foods. Following wheat, rice and corn, about our of total world's cereal production barley comprised of about 12% of with respect to grain production (FAO, 2009). While, in Pakistan, production of barley grain was 98,000 tones harvested during the crop year 2007-08 (GOP, 2008).

The barley contains substantially higher amounts of functional ingredient β-glucan. The use of β-glucan extracted from barley as human food due to its, positive role in human health has received a growing attention. The cell wall of barley and oat contains β-glucan, a non-starch polysaccharide composed of β-(1-4)-linked glucose units separated every two to three units by a single β-(1-3)–linked glucose, and referred to as a mixed linkage β-glucan (Carpita, 1996). β-glucan delays glucose absorption and regulates the level of blood glucose (Wood *et al*., 1994). The viscous nature of β-glucan physically slows glucose absorption in the gut. This property of β-glucan may be useful in the formulation of food products targeting management of diabetes.

The barley grains can be used to enhance the flavor, texture, appearance, and nutritional composition in a variety of functional foods, including hot cereals, cookies, crackers, breads, tortillas, granola bars, fruit-filled cereal bars, extruded snacks, and pastas and development of different beverages (Arndt, 2006). These beverages may enrich diet and improve human health because of its ease of consumption along with a usual meal. Barley β-glucan assume to be well suited for such an functional application, being capable of imparting a smooth mouth feel to beverage products and providing an excellent source of soluble dietary fiber. A barley β-glucan gum, with similar functional properties, could potentially serve as an alternative to traditional beverage thickeners such as alginates, pectin, xanthan, and carboxymethylcellulose (Giese, 1992).

The present study was planned to extract the β-glucan from Pakistani barley variety (Haider-93) and utilize it for the development of functional beverage, keeping in view the potential of barley β-glucan against glycemic index and functional importance of beverage the research was conducted to evaluate the functional and sensoric properties of barley β-glucan from Pakistani Barley variety.

Materials and Methods

Preparation and analysis of barley flour

Barley flour was prepared from Barley (cv. Haider-93) by grinding barley grains through UDY cyclone mill (mesh size 20 mm). The barley flour was analyzed for moisture, crude fat, crude protein, ash, crude fiber and NFE by following AACC (2000) methods.

Extraction and purification of β-glucan

β-glucan gum was extracted from barley flour by following with the method of Wood *et al.,* (1978) with some modifications. The barley flour (50 g) was suspended in 500 mL water, pH was adjusted to 10 with Na_2CO_3 (20%, v/w) and stirred vigorously for 30 min at a temperature of 45ºC. The mixture was centrifuged (Model 3K30, Sigma, Germany) at 15000 x g at 4ºC for 15 min. The supernatant was adjusted to pH 4.5 with 2M HCL and centrifuged again (20 min at 21000 x g at 4ºC) to separate precipitated protein which was discarded. The β-glucan was precipitated by adding of an equal volume of ethanol (99.9%) to the supernatant with slow stirring. The precipitate was recovered by centrifugation at 3300 x g for 10 min allowed to settle overnight at 4ºC and dried in a vacuum drier (Model: DZF 6020 R-A-alpha M). The extracted β-glucan was stored as pellets in high density polyethylene bags at 50 ^oC for further studies.

Analysis and utilization of β-glucan

The purified β-glucan pellets were analyzed for proximate composition, total dietary fiber (TDF), soluble and insoluble dietary fiber (SDF & IDF) as described by AACC (2000). The purified β-glucan was utilized in different formulations for the preparation of functional beverages, as given in Table I.

Preparation and Evaluation of β-glucan Beverage

The β-glucan beverage was prepared following the formulation of Temelli *et al.,* (2004) with some modifications (Table 1). The functional beverage samples were then organoleptically evaluated for sensory parameters such as color, flavor, sweetness, sourness and overall acceptability by a panel of five judges. The nine point hedonic scale was employed for the evaluation of samples.

Table 1. Treatment plan for the preparation of beverages

Treatments	β-glucan (%)
T_1	0 control (0.2% pectin)
T_2	0.2
T_3	0.4
T_4	0.6
T_5	0.8
T_6	1.0

Physicochemical Evaluation of β-glucan Beverage

The methods described by Yu *et al.,* (2003) were used for analyzing color values of β-glucan beverage samples i.e. L* a* b* color space with Color Tech-PCM (USA). The acidity of beverage samples was determined by following the method given in AOAC (2000). The viscosity of functional beverages was measured as per AACC (2000) through Rion viscometer (Rion Tech., USA).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using CoStat-2003 software following the method as described by Steel *et al.* (1997). The Duncun Multiple Range (DMR) was used to determine the level of significance between samples.

Results and Discussion

Chemical composition of barley flour

The barley flour contained 11.65%, 2.31%, 6.75%, 2.22% and 77.07% crude protein, crude fat, crude fiber, ash and nitrogen free extract (NFE), respectively (Table 2), which corroborated the earlier findings for Canadian varieties by (Li *et al*., 2004). The dietary fiber of barley flour in the present study was found 4.11% soluble, 7.37% insoluble and 11.48% total dietary fiber. The β-glucan is a soluble dietary fiber component and is present in the highest amounts in the endosperm of barley.

Table 2. Chemical composition of barley flour

Component	(%) on dry weight basis
Crude protein	11.65±1.10
Crude fat	2.31±0.21
Crude fiber	6.75±0.59
Ash	2.22±0.19
NFE	77.07±5.50
β-glucan	4.87±0.39

Analysis of β-glucan

The oat and barley were found to be main sources of β-glucan as soluble dietary fibe (Bjorck *et al.,* 1990). The results of the present study indicated that β-glucan possessed 9.96%, 1.17%, 7.22%, 1.72% and 76.38% of crude protein, crude fat, crude fiber, ash and nitrogen free extract (NFE), respectively (Table 3) which arealso described similarly by Bhatty (1991) who demonstrated that barley bran β-glucan contains 3.3% ash content. The fat content in the β-glucan was found higher as compared to reported by Faraj *et al*., (2006) who found that β-glucan concentrate comprised of 0.05% lipids which might be due to less impurity of β-glucan extracted in the present study.

The contents of starch, SDF, IDF and TDF in the present study are also in consistent with the results described by Faraj *et al*., (2006) who found variation from 0.4- 1.43% in starch content of β-glucan in soluble dietary fiber (SDF) range from 71.81–75.75 % and the in insoluble dietary fiber content of β-glucan gum pellets are in the range of (8.77-17.3%).

Table 3. Chemical Analysis of β-glucan

Component	(%)
Moisture	3.55±0.29
Crude protein	9.96±0.89
Crude fat	1.17±0.08
Crude fiber	7.22±0.55
Ash	1.72±0.14
NFE	76.38±6.99
Soluble dietary fiber	75.05±5.88
Insoluble dietary fiber	10.25±1.02
Total dietary fiber	85.30±6.79
Pentosans	2.63±0.19
Starch	1.90±0.17
β-glucan	4.87±0.39

Physicochemical evaluation of β-glucan beverages

The color values of beverage samples presented in Table IV indicated that the L*-value (color index) of functional beverages increased significantly as the level of β-glucan increased in the formulation of different

beverages. The beverage of T6 containing 1.0% β-glucan showed the highest L*-value (21.28) and followed by control beverage (without β-glucan) which got L*-value 19.69. However, the beverage of T5 containing 0.8% β-glucan gave the highest a*-value (1.65) and the lowest a*-value (2.27) was given by T1 control beverage (without β-glucan). Similarly, b*-value was significantly affected by treatments. The beverage T1 contains 0.2% pectin possessed the highest b*-value (10.80) followed by the beverage T6 contains 1% β-glucan and significantly the lowest b*-value was recorded in the beverage of T2 (0.2% β-glucan).

The present study indicated that incorporation of β-glucan resulted in improvement of beverages color as compared to the control beverage which was prepared by the addition of 0.2%pectin without addition of β-glucan. A small amount of precipitate was visible at the bottom of the β-glucan beverage which is due to insoluble protein and fiber components present in the β-glucan at low levels. Thus the precipitation of this material in case of β-glucan supplemented beverage might be a cause of higher L*-value for these treatments of beverage. In the present study a*-value decreased significantly by increasing the level of β-glucan in the beverages, which indicated that increased β-glucan concentration resulted in a less reddish product as compared to the control beverage.

The viscosity of beverages improved significantly due to the incorporation of β-glucan in beverages. The highest viscosity (21.75 mPa-s) was found in beverages of T6 containing 1% β-glucan followed by T5 beverage containing 0.8% β-glucan (Table 4). Total acidity varied significantly as a function of treatment. The variation in acidity in the present study was due to the degradation of sucrose, high fructose corn syrup and β-glucan by the action of microorganisms which causes production of acids in beverages (Renuka *et al.,* 2009).

Table 4. Effect of β-glucan incorporation on organoleptic evaluation of beverage

Treatments	Acidity	Viscosity	Color Values		
			L*	a*	b*
T_1 (0% β-glucan)	1.50±0.04	4.43±0.09	19.69±0.48	2.27±0.07	10.80±0.32
T_2(0.2% β-glucan)	1.49±0.03	7.01±0.17	20.12±0.51	1.40±0.05	9.62±0.25
T_3(0.4% β-glucan)	1.53±0.04	12.17±0.24	20.26±0.33	1.47±0.05	9.84±0.21
T_4(0.6% β-glucan)	1.53±0.06	16.37±0.49	20.43±0.37	1.28±0.04	10.08±0.17
T_5(0.8% β-glucan)	1.52±0.05	19.52±0.55	21.26±0.41	1.65±0.03	10.03±0.29
T_6(1% β-glucan)	1.53±0.04	21.75±0.76	21.28±0.43	1.35±0.03	10.55±0.22

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

Sensory evaluation of β-glucan beverages

The scores assigned to the sensoric attributes of 0.2% of β-glucan containing beverages revealed that these beverage got significantly the higher color scores (6.84) followed by the control beverage (0.2% pectin). Similar trend was observed in case of sweetness and sourness, however, the scores assigned to beverage prepared by the incorporation of 0.4% β-glucan got the highest score for flavor (Table 5). Contrarily the control treatment (0.2% pectin) got the top position regarding overall acceptability of beverage, followed by beverage samples prepared by incorporation of 0.2% β-glucan; with no significant differences in both treatments.

β-glucan's ability to increase viscosity upon addition to water makes it an excellent thickener for beverage applications. These characteristics are more appealing to the panelists for making decision about the overall acceptability of beverages. The results further indicated that in the beverages fortified with polysaccharides like β-glucan the quality characteristics of the beverages varies non significantly but it was also revealed that the incorporation should be not more than 0.4% of β-glucan. The further increase in β-glucan level thickens the beverage and higher consistency not appeals the consumer regarding sensory prospective of beverage.

Table 5. Effect of β-glucan incorporation on organoleptic evaluation of beverage

Treatments	Color	Flavor	Sweetness	Sourness	Overall acceptability
T_1 (0% β-glucan)	6.63±1.08	7.29±1.13	6.74±1.18	6.43±1.09	7.26±0.81
T_2(0.2% β-glucan)	6.83±1.23	7.37±0.58	6.69±0.63	6.40±1.11	7.31±0.93
T_3(0.4% β-glucan)	6.57±0.93	7.54±0.53	6.17±1.17	6.37±0.66	7.00±0.86
T_4(0.6% β-glucan)	6.03±0.90	6.60±1.09	5.89±0.91	6.14±0.92	6.43±0.78
T_5(0.8% β-glucan)	5.00±0.92	6.00±0.91	5.11±0.44	5.51±0.88	5.37±1.21
T_6(1% β-glucan)	4.94±0.73	5.86±0.66	5.03±0.71	5.11±0.72	5.37±0.73

Means carrying same letters within a column or row do not differ significantly ($P < 0.01$)

Conclusion

Incorporation of β-glucan has an effect on physicochemical characteristics and sensoric of beverage. The beverage improved regarding most of the physicochemical and sensory characteristics of the beverage. The acidity and viscosity of beverage improved linearly as the incorporation level of β-glucan increased in beverage formulation. Similarly, the consumers like more viscous beverage which was prepared by incorporation of β-glucan. However, the beverages containing lesser than 0.8% β-glucan were the least acceptable by the panelists. Further research is needed to know the thermal stability of β-glucan and its behavior with other food ingredients in beverages application to make stable foods.

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Effects of microbial transglutaminase on the quality of wheat bread supplemented with hull-less barley flour

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Abstract

Fortifying bread with hull-less barley flour reduces bread sensorial properties, though using microbial transglutaminase (MTG) to improve the quality of hull-less barley bread, has not been investigated. In this study, the impact of MTG on the quality of wheat bread supplemented with 20, 35 and 50% of hull-less barley flour was investigated. The MTG was added at five different levels (0, 0.5%, 1%, 1.5% and 2%; W/W). To increase the concentration of lysyl residues and possibly enhance the extent of cross-linking of protein matrix by MTG, a commertial soy protein isolate (SPI) was added at a level of 3%(w/w) in combination with MTG. Bread samples were evaluated in terms of their loaf volume, color characteristics, and firmness during 72 h. High cross- linking effect of MTG on wheat proteins significantly decreased the loaf volume of control wheat breads. Though substitution of wheat flour by hull-less barley flour decreased the loaf volume, but increasing levels of MTG at higher substitution levels (35 and 50%) could increase the loaf volume due to dilution effect of barley flour on wheat gluten proteins. Moreover the addition of SPI along with MTG had improving effects on the bread loaf volume and in all the samples MTG addition produced a lighter bread. The values obtained for firmness of MTG treated barley breads after 72h were lower than those of their sespective contents. Therefore, MTG could be incorporated in the dough formulation to improve hull-less barley bread masking quality.

Introduction

Hull-less barley (HB) has been investigated in many countries for use in feed, food, and industry, since the publication of the last review in 1986. Interest in the utilization of hull-less barley in the food industry developed largely due to its high β – glucan content. Cereal β – glucan is a soluble fiber found primarily in barley (3-11%) and oats (3-7%) (Bhatty 1992; Skendi et al 2003). There are a number of health benefits attributed to the consumption of β – glucan including better regulation of blood glucose and insulin levels (Wood et al 2000; Panahi et al 2007; Kim et al 2009) and the lowering of blood cholesterol levels (Behall et al 2004; Karmally et al 2005; Naumann et al 2006; Queenan et al 2007). Cholesterol reduction and glucose regulation are important factors in the prevention of heart disease and type 2 diabetes (Canadian Diabetes Association 2007; Heart and Stroke Foundation 2007). However, incorporation of large amounts of barley or oat flour needed to fortify bakery products, can lead to quality defects, a reduction in product sensory parameters (Skribic et al 2009), so using microbial transglutaminase (MTG) can successfully incorporate in the dough formation to improve bread making quality. Microbial Transglutaminase (MTGase) (EC 2,3,2,13) catalyzes protein cross-linking through the formation of inter- or intramolecular ε – (γgln)- lys isopeptidic bonds. These bonds cause the homologous and heterologous polymerization of proteins (Motoki and Seguro 1998). Sakamoto et al (1996) found that treatment of noodles and pasta doughs with TGase prevented the deterioration of texture upon cooking and improved the strength of the products. TGase improved dough elasticity and crumb strength of bread baked from dough containing TGase (Gerrard et al 1998), because TGase transformed weak gluten into strong gluten through its effect on rheological behavior (Larre et al 2000). Even at a low TGase level, the quality of bread made with weak flour could be significantly improved by polymerization of gluten (Larre et al 1993; Basman et al 2002). The improving effects of TGase on bread, noodle, and pasta-making, and other food processing such as milk, meat, and fish have been reported but not on the hull-less barley bread. Little information is available on the effects of MTGase on hull-less barley bread. Therefore the objectives of this study were to investigate the main reasons of improving effects of MTG on breadmaking. In this study the effects of

MTG on physical properties of the wheat bread supplemented with hull-less barley flour produced under pilot plant settings was investigated. In addition, a commercial soy protein isolate (SPI) in combination with MTG (MTG+SPI) was used to study its effects on wheat and hull-less barley bread quality. Quality of the final products was evaluated by breadmaking with the resultant measured values of volume and specific volume of bread by the seed replacement method, crumb firmness by a texture analyzer (instron) and color properties by specrtophotometer chroma meter.

Materials and methods

Materials

Grains from two Iran grown wheat and hull-less barley cultivar, Zarrin and EHDS 18 were milled in to flour and the wheat flour sieved with a 100-mesh sieve. Zarrin is a cultivar that represent strong dough properties. Dry yeast, sugar, salt and improving agents (gluten, ascorbic acid) were purchased from a local bakery store. Microbial transglutaminase with an activity of 100 U/g was a gift from Ajinomoto Co. Inc and Soy Protein Isolate (SPI) was obtained from sole company (India).

Bread preparation

Bread was prepared under pilot plant conditions. All dry ingredients were mixed together and the amount of water used to hydrate the dry ingredients was determined using a farinograph (Brabender Farino/Resistograph) equipped with a 50-g mixing bowel. The temperature was maintained at 30 ºC, and the optimal amount of water addition was determined as the point at which the consistency of the sample reached 500 BU. Then, all ingredients were combined and mixed in a mixer (model Hobart, Germany) for 15 min until an adequate dough was formed. The prepared dough was split into pieces of 40 g. the loaves were then fermented for 2 hr in a fermentation cabinet (model Esfahan sanat, Iran) maintained at 30ºC and 82% relative humidity. After fermentation, the dough was baked in an oven (model Esfahan Pokht, Iran) for 15 min at 220 ºC and then cooled on wire racks for 30 min. The dough samples were made from wheat flour supplemented with 20, 35 and 50% of hull-less barley flour treated with 0.5%, 1% , 1.5%, 2%, 1% MTG+ 3% SPI and 1.5% MTG + 3% SPI.

Table 1. Recipes for bread dough

Ingredient (%)	WB20*	WB35**	WB50***
Wheat flour	80	65	50
hull-less barley flour	20	35	50
sugur	15	15	15
salt	15	15	15
yeast	1.2	1.2	1.2
improving agent	0.4	0.4	0.4

* WB20: Wheat Bread with 20% of hull-less barley flour
** WB35: Wheat Bread with 35% of hull-less barley flour
*** WB50: Wheat Bread with 50% of hull-less barley flour

Loaf characterization

Loaf volume of the experimental dough was determined by the seed replacement method. The color of the loaves was analyzed using a colorimeter (model data color,U.S.A) in terms of L values. Crumb firmness was determined on loaves at the times 0, 24, 48 and 72hr, by performing compression tests using a texture analyzer (Instron, model 1140, England). A bread crumb cylinder (20 mm long, 25 mm diameter) was cut from each bread and compressed once to 50% of its original height at a crosshead speed of 100 mm/min. Compression force for each sample was recorded as a measure of firmness.

Statistical analysis

Analyses of variance were performed by ANOVA test, and significance of differences between the means was determined by Duncans new multiple range test ($p \leq 0.05$) (Steele and Torrie 1980).

Results and discussion

Loaf characterization

The loaf characteristics of the experimental bread are presented in table I. The bread with 50% of hull-less barley flour, had the lowest volume ($P \leq 0.05$), while the wheat bread had the highest one. Cavallero et al (2002) and trogh et al (2005) reported that the addition of barley flour and high β-glucan fractions from barley also decreased the volume of breads in comparison to a wheat control. β-glucan has the ability to bind large amounts of water and by increasing the amount of water-extracted β-glucan concentrate added to bread, Jacobs et al (2008) reported a progressive decrease in loaf volume, that is likely resulted from the binding of the water needed for gluten development by β-glucan (Gill et al 2002).

The breads with 50% of hull-less barley flour were more firm than the control bread. Jacobs et al (2008) and Gill et al (2002) reported similar trends of increased firmness upon addition of a fiber-rich fraction with β-glucan and barley flour, respectively, to bread. This was attributed to β-glucan binding tha water needed for gluten development and steam generation, thus resulting in a reduction in loaf volume and a subsequent increase in firmness (Gill et al 2002).

In terms of the color, the bread darkened ($P \leq 0.05$) upon each increase in hull-less barley flour level. These trends were in agreement with Moriartey et al (2010).

Table 2. Characteristics of bread with varied level of β-glucan

	β-glucan (%)	**Volume (mL)**	**Firmness (kgF)**	**Color (L, Crust)**
Control	0	86.19 ± 1.17^{a}	357 ± 13.3^{c}	54.29 ± 0.54^{a}
With β-glucan	20	80.98 ± 1.31^{b}	461 ± 12.2^{b}	47.31 ± 0.89^{b}
With β-glucan	35	73.66 ± 1.27^{c}	462 ± 13.3^{b}	47.25 ± 0.76^{b}
With β-glucan	50	42.6 ± 1.11^{d}	720 ± 14.3^{a}	40.11 ± 0.5^{c}

Values in the same column with the same letter are not significantly different ($P \geq 0.05$)

Effects of MTG on the loaf volume

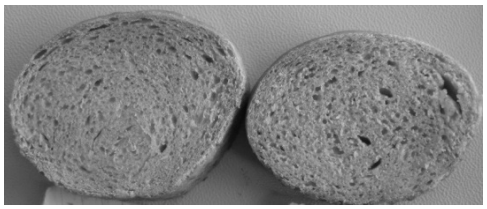
MTG cross links wheat proteins as suggested by the decrease in the free amino groups. This cross-linking results in a dough with an improved elastic and viscous behavior. The improvement in the viscoelastic properties of the dough was associated with an improvement in the ability of flour to retain the carbon dioxide produced during proofing, resulting in wheat bread with higher volume and crumb strength (singh gujral, rossel, 2004). Research by Basman et al (2002) found a decrease in loaf volume for wheat bread with the addition of MTG. similar result were found with our research. In wheat bread and the breads with 80% of wheat flour, by addition of MTG, the volume of the control samples, decreased, which was probably due to excessive cross linking within the dough system. This coincides with research by Gerrard et al (1998), where these authors detected a strong increase in crumb strength for wheat bread due to the addition of MTG. Crumb grain of breads was regular and elastic when low concentration of MTG was used. Higher MTG concentrations led to a more compact crumb, so the loaf volume decreased. The use of MTG with a flour of a wheat cultivar of high baking performance, leads to a decreased loaf volume, because the ε – (δ gln)- lys cross-links strengthen the dough in such a way that the gas produced by the yeast is not able to extend the dough. The use of MTG in flours producing sticky, poorly extensible doughs with weak gluten would probably lead to an increased loaf volume because of the the formation of ε – (γ gln)- lys cross links which would improve dough properties with respect to dough cohesion, gluten strength, and gas holding capacity. Due to gluten strengthening the dough layers are more stable and therefore less permeable for the gas enclosed between the layers. So MTG might be used to improve the quality of flours with low bread making performance (Nicole bauer, 2003). Similar results were found with our research. Addition of MTG had significant effect on the loaf volume. For samples with 35 and 50% of hull-less barley flour, loaf volumes generally increased up to 0.5, 1 and 1.5% MTG addition levels, and at 1.5% MTG the maximum volume achieved. The increase in loaf volume might be the result

of MTG transforming a weak gluten in to a stronger one. This was also reported by Larre et al and koksel et al. Gerrard et al reported that MTG might produce beneficial effects during bread making that are similar to those of oxidizing improvers. However in the present study a negative effect on loaf volume was observed at higher MTG levels (2%) probably due to excessive cross linking causing an over strong dough, and it is due to the formation of cross linking in proteins and in this state, the volume would decreased.

Table 3 Effects of MTG on the volume of breads

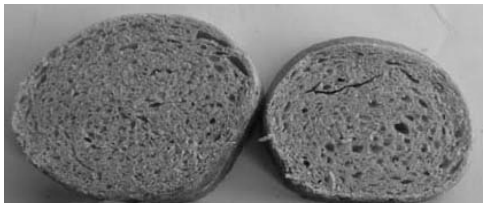
	W	**WB20**	**WB35**	**WB50**
Control	60.6 ± 1.2[a]	60.9 ± 3.1[a]	44.25 ± 3.3[c]	52.15 ± 3.8[c]
MTG (%)				
0.5	60.25 ± 1.4[a]	60.15 ± 3.2[a]	49.15± 4.3[c]	50.8 ± 0.3[c]
1	54.6 ± 2.3[c]	55.8 ± 1.5[c]	53.25± 2.4[c]	53.4 ± 3.2[c]
1.5	54.8 ± 0.4[c]	55.3 ± 3.4[c]	55.35 ± 3.53[c]	54.1 ± 2.4[c]
2	51.4 ± 3.12[c]	53.1± 2.6[c]	50.1±3.9[c]	53.25 ± 3.6[c]
MTG + SPI (%)				
1+ 3	53.3± 2.5[c]	54.1± 2.4[c]	57.35 ± 5.23[c]	56.4 ± 2.5[c]
1.5 + 3	51.1 ± 3.6[c]	53.35± 2.7[c]	59 ± 2.6[b]	59.05 ± 2.6[b]

Values in the same column with the same letter are not significantly different (P≥0.05)
W: Wheat Bread
WB20: Wheat Bread with 20% of hull-less barley flour
WB35: Wheat Bread with 35% of hull-less barley flour
WB50: Wheat Bread with 50% of hull-less barley flour



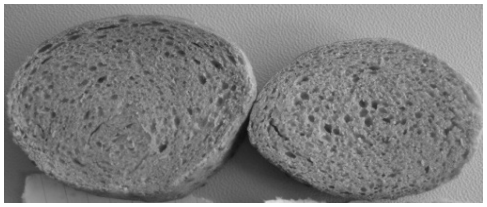
Control 1.5% TG

Figure 1. Effect of MTG on the volume of WB 20



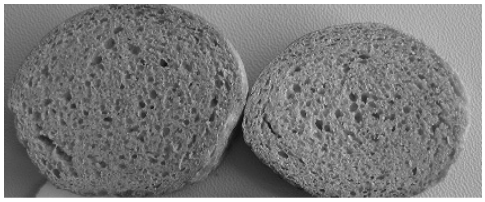
1.5 %TG+3% SPI control

Figure 2. Effect of MTG on the volume of WB35



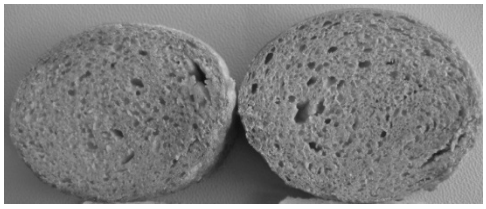
Control 1%TG+SPI

Figure3-Effect of MTG on the volume of W



0.5% TG 1%TG+SPI

Figure 4 -Effect of MTG on the volume of WB20



Control 1%TG

Figure 5- Effect of MTG on the volume of WB50F

1.5%TG+ SPI control

Figure 6- Effect of MTG on the volume of WB50

Effect of MTG on bread firmness

As illustrated in the present work, MTG formed cross links within the gluten system. Studies by Gerrard (2002) showed that the rate of protein cross linking through MTG depends both on the particular protein structure and the disposition of lysyl and glutamine residues. MTG has the ability to form crosslinking networks in gels with soy protein. Research by kuraishi et al (1996) has also shown that for gels, MTG has an impact on the water holding capacity. It is presumed that the protein network formed by MTG has the ability to trap water and hence, cause an increase in the water holding capacity and decrease in staling.

Table 4 Effects of MTG on firmness of breads

	Firmness			
	0 (h)	**24 (h)**	**48 (h)**	**72 (h)**
Control				
W	402.5^{k}	467.5^{l}	552.5^{h}	602.5^{g}
WB20	431^{k}	541^{j}	732.5^{d}	701^{e}
WB35	502.5^{i}	541^{j}	711^{d}	771^{d}
WB50	706^{d}	742.5^{c}	806^{b}	892.5^{a}
0.5% MTG				
W	701^{d}	740.5^{c}	761^{c}	801.5^{c}
WB20	532^{i}	561.5^{i}	741^{d}	771^{d}
WB35	481^{j}	501^{k}	601^{g}	771.5^{d}
WB50	661^{e}	641^{f}	724^{d}	731^{e}
1% MTG				
W	721^{c}	781^{b}	781^{c}	801^{c}
WB20	541^{i}	601.5^{g}	831^{b}	760.5^{d}
WB35	441^{k}	501^{k}	701^{d}	701^{e}
WB50	661^{e}	661^{e}	721^{d}	736^{e}
1.5% MTG				
W	678^{e}	761^{b}	841.5^{c}	812.5^{c}
WB20	513.5^{i}	532.5^{j}	891.5^{a}	731^{e}
WB35	421^{k}	511^{k}	821^{d}	703^{e}
WB50	561^{h}	581^{h}	641^{f}	661.5^{f}
2% MTG				
W	751^{b}	811^{a}	781^{b}	852.^{5b}
WB20	601^{g}	601.5^{g}	880.5^{a}	820.5^{c}
WB35	503.5^{i}	611^{g}	701^{b}	852.5^{b}
WB50	771^{a}	771^{b}	871.5^{a}	851^{b}
1% MTG +3% SPI				
W	601^{g}	781^{b}	781^{c}	801.5^{c}
WB20	521.5^{i}	602.5^{g}	802.5^{b}	771.5^{d}
WB35	411.5^{k}	511^{k}	651^{f}	721^{e}
WB50	541^{i}	561.5^{i}	681.5^{e}	701^{e}
1.5% MTG + 3% SPI				
W	620^{f}	701.5^{d}	761^{c}	781^{d}
WB20	531^{i}	601^{g}	810.^{5b}	716^{e}
WB35	401.5^{k}	506^{k}	613.5^{g}	711.5^{e}
WB50	561^{h}	501^{k}	631^{f}	652^{f}

Values in the same column with the same letter are not significantly different (P≥0.05)
W: Wheat Bread; WB20: Wheat Bread with 20% of hull-less barley flour; WB35: Wheat Bread with 35% of hull-less barley flour; WB50: Wheat Bread with 50% of hull-less barley flour

Effects of MTG on bread color

In the present study, color characteristics of bread samples measured in Hunter color system and expressed as L (brightness) is shown in table. Addition of MTG generally led to improving effects on crumb color in all the breads. It is also worth noting that crust color became progressively lighter as the enzyme level increased. In all the breads the level of 1.5% MTG+ SPI addition had the lightest color. The lighter crust color might be the result of a limited amount of maillard reaction due to a decrease in the

amount of available lysine because of MTG reactions. Basman et al (2002a) found that the color of bread crust became lighter due to MTG treatment that was attributed to the lower intensity of Maillard reaction. Furthermore, the release of ammonia during the MTG-catalyzed cross-linking reaction (Motoki and Seguro, 1998) might also participate in the Maillard reaction and thus contribute slightly to the changes in color properties (Wu and corke, 2005). Since the color characteristics reported here are the results of color reflectance measured by colorimeter, it can be contemplated that apart from the suggested chemical mechanisms, cross-linking by MTG might also have influenced the physical structure of bread thereby affecting the reflectance properties.

Table 5 Effects of MTG and MTG+SPI on color characteristics of breads

	Crust				**Crumb**			
	W L*	WB20 L*	WB35 L*	WB50 L*	W L*	WB20 L*	WB35 L*	WB50 L*
Control	54.29	47.31	47.25	44.11	66.23	61.24	47.2	59.19
MTG (%)								
0.5	56.03	47.29	59.12	40.03	66.18	62.07	62.17	53.38
1	54.19	59.15	59.02	55.43	64.27	62.35	63.09	65
1.5	63.21	53.47	61.16	63.25	61.22	62.28	61.4	65.25
2	64.28	56.25	60.22	62.43	62.38	63.10	62.06	65.32
MTG+SPI (%)								
1 +3	65.25	57.3	62.01	65.11	59.22	61.19	62.04	64.43
1.5 + 3	65.12	59.24	65.12	60.43	58.22	63.14	64.3	67.27

Values in the same column with the same letter are not significantly different (P≥0.05)
W: Wheat Bread
WB20: Wheat Bread with 20% of hull-less barley flour
WB35: Wheat Bread with 35% of hull-less barley flour
WB50: Wheat Bread with 50% of hull-less barley flour
L*= brightness

Conclusion

Measurement of the hull-less barley bread´s physical properties indicated that hull-less barley flour addition at the 50% level decreased loaf volume, increased firmness, and resulted in darker bread. We report here in the beneficial effects of MTG in bread making. The MTG enzyme shows great promise as a processing aid in the bulk manufacture of bread. Our results indicated that addition of MTG to different flours influenced their properties in different manner. At lower MTG addition levels the quality of breads made with a weak flour can be improved to a quality level that might be achieved with stronger flour. The improving effects were lower for wheat flours and breads with 80% of wheat flour. Optimum enzyme level should be estimated for any given flour sample to obtain maximum bread loaf volume and minimum crumb firmness values. This optimum level will of coarse depend on the flour quality of the sample itself. Formation of significant numbers of covalent crosslinks by MTG can be successfully incorporated in the baking formula, which may minimize the cost of MTG utilization in the bread baking industry.

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The influence of vital wheat gluten addition on the quality of baking products

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Vital wheat gluten is used to improve and stabilize flour quality, especially if the flour is achieved through milling grains of low baking features. What is more, vital wheat gluten helps increase the flour's water absorption by ca. 10 – 15% which results in heightened efficiency of dough and baking goods.

The conducted research investigated the influence of vital wheat gluten addition on the quality of baking products (mainly nutritional value, determined with the aid of the chemical score factor). After having selected the desired type of flour with the required baking parameters, optimal doses of gluten were determined so as to ensure a positive influence on the baking and nutritional properties of flour and, further, to achieve an improved quality of baking products. The following discriminants were evaluated: - overall appearance of the baking - bread crust, its colour and thickness - porosity, elasticity and other features of pith. This facilitated the proper quality classification of the derived bread samples according to the adequate PN norm. Gluten addition amounted to, respectively, 5, 10, 15 and 20%; the 15% addition proved most optimal. Bread produced from baking in standard conditions was categorized as belonging to the top quality class. It had the highest volume (3581 cm^3), its porosity amounted to ca. 78%, and its Cs factor value was increased by 10% (Cs for lysine as limiting aminoacid: 52%, and the FAO pattern: 46,8%). Thus, the investigation has proven that in this way the overall nutritional value of baking has increased.

Gluten addition to baking doughs significantly improves their reological qualities and prolongs their developmental processes, as well as enhances doughs' resistance to kneading, reducing softening. Such doughs are characterized by a reduced viscosity, heightened susceptibility to mechanical processing (due to reduced adhesion), along with an increased resistance to tearing processes. Gluten addition leads also to an improved propensity for producing and maintaning gases in the dough, which in turn yields baking goods of an increased volume (by 5-25%), of a more delicate and elastic pith, and of an extended expiry dates.

Effects of white cabbage powder on cookie quality

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Demand for health oriented products which have high fibre and natural antioxidant, sugar-free and low calorie products is increasing because of their beneficial effects to overcome health problems such as cancer, cardiovascular diseases, hypertension, diabetes. Vegetables are good sources of natural antioxidants and dietary fiber. Among vegatables white cabbage have been used for years in human nutrition due to its high antioxitant, phenolic compound, dietary fiber, mineral capacity and low calori content.

The aim of this study was to evaluate the effects of dehydrated white cabbage powder (DWCP) supplementation on chemical, physical, nutritional and sensorial characteristics as well as the consumers' acceptance and purchase intent of the cookies.

Cookie samples were prepared with blends containing 0–2.5-5.0 and 7.5% of DWCP substituted for wheat flour. Total dietary fiber and mineral (Calsiyum, potassium) content of cookies improved with increased amounts of DWCP. Total phenolic compounds, antioxidant activity, width, thickness spread ratio and surface cracking did not differ significantly ($p < 0.05$) among the cookies. While color values (L and b) of DWCP cookies were increased, b values were decreased when compared with control samples. Cookies with 2.5 % substitution level of DWCP showed the highest scores for sensorial attributes, consumers' acceptance and purchase intent.

From these results, it is considered possible to use DWCP containing cookies to increase their functional properties. Also substitution level of DWCP can be further raised by using some aroma compounds to suppress unwanted smell and flavour of cabbage.

Effect of process parameters on polygalacturonase production by mutant strain of *Aspergillus sojae* in serial bioreactor system

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Among fungal enzymes, pectinases are associated with development, fruit ripening and degradation of the pectic substances in the vegetal cell wall. This degradative process plays an important role in food technology, due to reduction in time of filtration and to the volume increase, and juice clarification. This process leads to a more stable and concentrated product. The total value of the food and beverage enzyme market is estimated to reach $320 million by 2013. Polygalacturonase (PG) attracts the most attention among the family of pectinolytic enzymes. The main sources of the pectinolytic complex enzymes are yeast, bacteria and a large variety of filamentous fungi, for which the most relevant ones are *Aspergillus*.

In our preliminary studies we produced polygalacturonase (PG) by mutant *Aspergillus sojae* ATCC 20235 in shake flask system and optimized an industrial low cost media formulation. Classical mutation and selection program was conducted by Bremen Jacobs University, to maximize product formation.

In this study it was aimed to use this optimized media formulation in batch mode 1 l scale serial bioreactor system in order to investigate the effects of agitation speed, aeration rate, pH and dissolved oxygen (DO) level on PG activity.

It was observed that maximum PG activity was achieved at 600 rpm agitation speed (120 U/ml), 1.5 vvm aeration rate (120.4 U/ml) and uncontrolled pH (93.48 U/ml) conditions. Besides the cascading experiment which was used to control the DO level, lead up to maximum PG activity (114.49 U/ml) when agitation and aeration cascade was used together.

Lyophilisation of traditional Latvian rye sourdough starters

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Abstract

Preparing of sourdough is one of the oldest biotechnological methods, but the research is still going on and is crucial. In Latvia the spontaneous sourdough starter is used in traditional dark rye bread baking which microflora is determined in flour and in microorganism cultures presented in external environment. The aim of current research was to analyze technological and biochemical parameters of fresh and lyophilisated (freeze dried) spontaneous sourdough starters. Spontaneous sourdough starters were prepared using two types of flour - 100% type 1370 rye flour (starter U) and 80% type 1370 rye flour with addition of 20% biologically active enzyme rich rye flour (starter M). Sourdough starters were lyophilisated using vacuum freeze drier „Armfield FT 33". Total titratable acidity and pH were measured in each preparation step of spontaneous sourdough starter to control the fermentations. Plate counting method was used for lactic acid bacteria detection before and after lyophilisation. Organic acids were determined using HPLC (Agilent 1200). Total titratable acidity calculated on dry matter dropped insubstantially after lyophilisation of sourdough starters compared to fresh sourdough starters after last stage of fermentation (2.3 – 3.5%). Acceptable amount of lactic acid bacteria calculated on dry matter was counted in fresh sourdough starters after 48h fermentation as well as in freeze-dried sourdough starters (10^7-10^8 CFU·ml^{-1}). Investigated spontaneous starters has preferable properties for further application in Latvian traditional rye sourdough fermentation and contain biologically active compounds which benefit technological process of rye bread baking. Addition of 20% biologically active enzyme rich rye flour in starter M accelarates metabolysm of lactic acid bacteria.

Key words: Sourdough, lyophilisation, lactic acid bacteria

Introduction

Classic spontaneous sourdough preparation is a multiple stage process that starts with a mixture of flour and water left for a specific period of time. Every next stage is prepared with fresh flour and water added to the previous stage. The character of the process results from the growth of microorganisms in different environmental conditions. Temperature, dough consistence and dough resting time at each stage determine development of active microflora (Muller et al., 2001). In addition to environmental influence, flour is largely responsible for the properties and quality of spontaneously fermented sourdough. Sourdough fermentation begins with aerobic growth immediately upon mixing flour and water. Once oxygen is depleted, anaerobic fermentation begins with the growth of lactic acid bacteria, which produces acids that enhance their rapid growth when the pH value has dropped too low for other microorganisms to develop. Thereby lactic acid bacteria become the most abundant microorganisms in the sourdough and they are therefore responsible for the final stages of the sourdough processing.

In the scientific literature data about grain biological activation and its application in bread production with the main purpose to increase ready product nutritive value is summarised. Grains on first stage of germination where biochemical processes are beginning (protein hydrolyses, synthesis of vitamins, production of essential amino acids) are called biologically activated grains. The content of dietary fibre, vitamins B_2 and E, and niacin increases and vitamin C synthesises during the grain activation. Such grains contain elevated amount of enzymes which are beneficial for sourdough fermentation (Rakcejeva and Skudra, 2006).

Scientific publications show that application of spontaneous sourdough in rye bread production may cause unstable quality of rye bread (Reed and Nagodawithana, 1995). To ensure the stability of rye bread

production process selected lactic acid bacteria starter cultures are used in European bakeries to provide controlled sourdough fermentation. Usually starters are frozen or freeze dried mother sponges or freeze-dried lactic acid bacteria concentrates. These preparations are very stable over time and have a shelf life of 3 months at -4 °C and 1 year at -20 °C without oxygen. Their purpose is to make a full sourdough in a single stage. The strains generally used are *L. plantarum, L. brevis, L. sanfrancisco,* and *L. casei*. The amount used is normally 0.1% of flour weight in the sourdough. Fermentation time is 16–24h at temperatures between +25 °C and +30 °C (Poitrenaud, 2003). However lactic acid bacteria starters selected in Europe frequently does not satisfy demands of Latvian bakers.

Since the absence of locally produced ready to use sourdough starters and lactic acid bacteria pure cultures the aim of this research is to analyze technological and biochemical parameters of fresh and lyophilisated (freeze dried) spontaneous sourdough starters.

Materials and methods

Spontaneous sourdough starters were prepared using two types of rye flour - 100% (type 1370, ash content 1.45%) - **starter U** and 80% (type 1370, ash content 1.45%) with addition of 20% biologically active enzyme rich rye flour - **starter M**. 48- hour three stage fermentation process was used for spontaneous sourdough preparation with two refreshings after 24 and 32 hours (Figure 1).

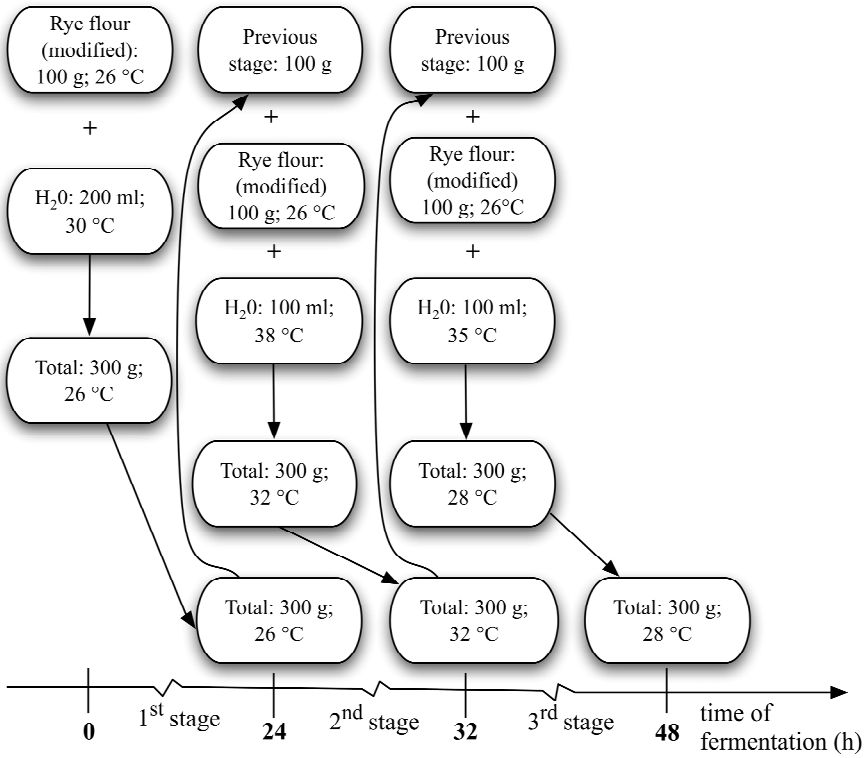

Figure 1. Technological process of three-stage spontaneous rye sourdough fermentation (Kozlinskis et. al., 2008)

Starters were lyophilisated using vacuum freeze drier "Armfield FT 33" for 48 hours at -55 °C. Total titratable acidity and pH according to "Standard - Methoden fur Getreide, Mehl und Brot" were measured in each preparation step of spontaneous starter to control the fermentations (Spicher, Stephen, 1993). Plate counting method ac according to standard method ISO 9332:2003 was used for lactic acid bacteria detection before and after lyophilisation using MRS agar (Scharlau, Ref. 01-135). Organic acids according to modified standard method AOAC 986.13 were determined using HPLC (Agilent 1200). Dry matter of samples was analysed according to standard LVS 272:2000 using humidity scales KERN MRS-120-3.

Sourdough refreshment was accomplished before testing the lyophilisated sourdough starters in bread production process using 10% sourdough U or M, 100% rye flour (type 1370, ash content 1.45%), 70% drinking water and fermented for 18 hours at +28 °C. Dough fermentation was accomplished using 100% refreshed sourdough, 100% rye flour (type 1370, ash content 1.45%), 80% drinking water fermented for 2 hours at +32 °C. Fermented dough was baked at +220°C for 30min using rotary-convection oven SvebaDahlen S-400.

Results and discussion

The content of organic acids has a significant role in taste and aroma production of rye bread. To characterize the relation between organic acids in sourdough, a molar ratio of lactic acid to acetic acid – the fermentation quotient (FQ) is widely used. For preparing sourdough bread with balanced taste properties fermentation quotient in sourdough should be 4. If insufficient amount of acetic acid is produced in sourdough the taste of a product is weak and indistinctive. While in the case of low FQ the taste of a product is too strong and acid (Spicher, Stephan, 1993).

The results of this study (Figure 2) show that fermentation quotient after 48 hour fermentation reaches 3.2 in sourdough U and 4.5 in sourdough M, confirming that sourdough M corresponds to requirements of high quality sourdough. The content of lactic acid during first stage of fermentation reaches 0.37 $mg \cdot g^{-1}$ in sourdough U and 3.05 $mg \cdot g^{-1}$ in sourdough M due to higher activity of lactic acid bacteria and availability of nutrients in biologically active enzyme rich rye flour. Content of acetic acid in sourdough U increases from 0.17 $mg \cdot g^{-1}$ to 1.38 $mg \cdot g^{-1}$ during 48 hour fermentation exceeding the amount of acetic acid in sourdough M where it reaches 1.04 $mg \cdot g^{-1}$ after the last stage of fermentation. It could be explained by higher activity of heterofermentative lactic acid bacteria in sourdough U compared to sourdough M where lactic acid producing homofermentative lactic acid bacteria are dominant after 48 hour fermentation.

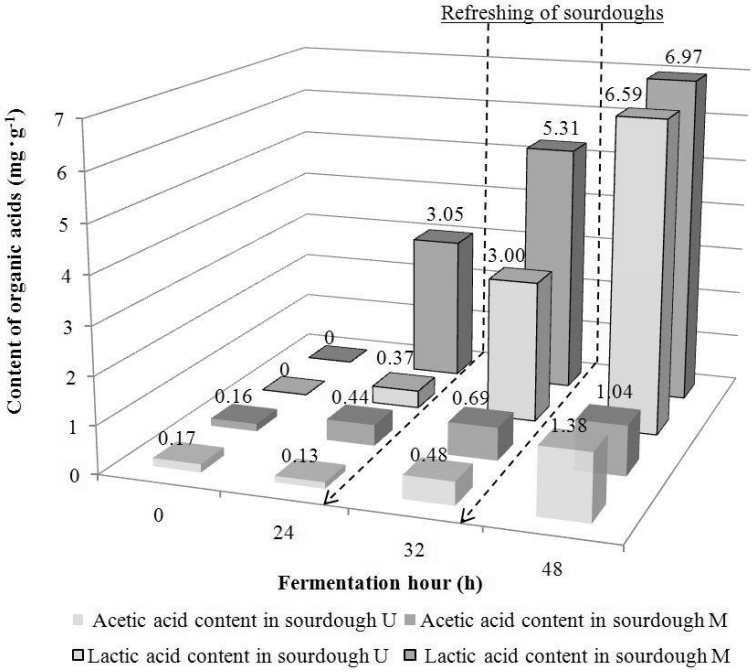

Figure 2. Content of lactic acid and acetic acid during three-stage spontaneous rye sourdough fermentation

Fresh sourdoughs U and M where lyophilisated after the last stage of fermentation and comparison of titratable acidity and lactic acid bacteria plate count calculated on dry matter is represented in Table 1. Total titratable acidity calculated on dry matter dropped insubstantially after lyophilisation of starters compared to fresh starters after last stage of fermentation (2.3% in sourdough U, 3.5% in sourdough M).

Regardless of decrease of lactic acid bacteria after lyophilisation – 68% in sourdough U and 57% in sourdough M acceptable amounts of lactic acid bacteria calculated on dry matter was counted in fresh sourdoughs after 48h fermentation as well as in freeze-dried starters (10^7-10^8 CFU·ml^{-1}). Lactic acid bacteria plate count in sourdough with addition of biologically active enzyme rich rye flour exceeds plate count in sourdough without additions in fresh form by 35% and in lyophilisated form by 83%. More resistant strains of lactic acid bacteria against dry-freezing are dominant in sourdough M which explains its excess over sourdough U.

Table 1. Parameters of fresh and lyophilisated sourdoughs calculated on dry matter

Sourdoughs	Dry matter (%)	Log 10 CFU·g^{-1}	Titratable acidity (ml 0.1 n NaOH)
Fresh sourdough U	49.82	7.83	14.90
Lyophilisated sourdough U	94.37	7.33	14.50
Fresh sourdough M	45.56	7.96	17.60
Lyophilisated sourdough M	96.15	7.59	17.00

After refreshing (activating) lyophilisated sourdoughs U and M (Table 2), pH and titratable acidity data show that microflora of sourdough M produces more acidity compared to sourdough U demonstrating identical trend as in previous experiments. After fermentation of dough using sourdough M its pH and titratable acidity exceeds the data presented by other authors investigating Latvian traditional rye bread baking technologies (Kunkulberga, Segliņš, 2010). Similarly acidity data of rye bread using both sourdoughs satisfies requirements of local market. Sourdough M show optimal results for applying it in traditional Latvian rye bread baking technology and possibly may substitute foreign commercial starters.

Table 2.Testing of lyophilisated sourdoughs in bread production process

Lyophilisated sourdough	pH	Titratable acidity (ml 0.1 n NaOH)	pH	Titratable acidity (ml 0.1 n NaOH)	pH	Titratable acidity (ml 0.1 n NaOH)
	After refreshing		After fermentation of dough		After bread baking	
U	4.17	16.9	4.18	11.2	4.79	5.8
M	4.03	18.1	3.85	12.0	4.61	7.0

Conclusions

Investigated spontaneous sourdough starters has preferable properties for further application in Latvian traditional rye sourdough fermentation and contain biologically active compounds which benefit technological process of rye bread baking. Addition of 20% biologically active enzyme rich rye flour in starter accelarates metabolysm of lactic acid bacteria and positively affects dough fermentation.

Acknowledgements

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Optimization of *Aspergillus niger* tannase production under solid state fermentation using statistical experimental designs

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Abstract

Tannin acyl hydrolase (TAH), also known as tannase is an enzyme with important applications in food, feed, pharmaceutical and chemical industry. However, the practical use of this enzyme is at present limited due to high production costs. The aim of this work was to optimize TAH production by *A., niger* GH1 in solid state fermentation. Tannase production optimization was carried out in glass column reactors using polyurethane foam as inert carrier. Optimization of production was done using a two stages strategy: First 10 variables (tannic acid, K_2HPO_4, $NaNO_3$, KCl, $MgSO_4$ and $FeSO_4$ concentration, incubation temperature, inoculum age, air flow rate and initial pH) were evaluated using a Plackett-Burman statistical design. From 10 screened variables, only three factors (tannic acid concentration, initial pH and temperature of incubation) significantly affected tannase production. These factors were optimized by a Box-Behnken statistical design. Maximum tannase production was reached at the highest tannic acid concentration (50 g/L), and the lowest initial pH (4.0) and temperature of incubation (30 °C). These results indicated that the GH1 strain of *A.niger* is adapted at extreme conditions of pH and tannin concentration. Using this statistical optimization method, tannase production was increased 1.97 times (from 4030 to 7954 U/L). Experimental designs applied in this work proved to be an efficient method for optimizing enzyme production in solid state fermentation.

Key words: tannin acyl hydrolase, optimization, packed bed bioreactor

Introduction

Tannase (EC 3.1.1.20) is an enzyme that catalyses the hydrolysis of the ester and depside bonds presents in gallotannins, complex tannins, and gallic acid esters (Aguilar *et al*., 2007). Tannase is mainly utilized in the elaboration of instantaneous tea and gallic acid production from natural sources. But, due its hydrolytic and synthetic properties, tannase has many other potential applications in chemical, pharmaceutical and food industries (Belmares *et al*., 2004). However, most of these applications have been missed due to the high production cost of the enzyme. Therefore, there has recently been great interest in improving the processes for tannase obtention.

In this regard, several authors have studied the production of tannase in solid state and submerged fermentation (SSF and SmF) and found several advantages of SSF system over the traditional SmF These advantages include the extracellular nature of enzyme and higher titers of production (Aguilar *et al*., 2001). Furthermore, in the last years a number of papers dealing with the optimization of SSF for the production of this enzyme have been published (Manjit *et al*., 2008; Paranthaman *et al*., 2010). In most of them optimization was carried out by the one factor at time method. This strategy is time consuming and requires a large number of experiments to determine the optimum levels. Also, is unable to detect the interaction among the variables and is therefore unreliable. Thus optimization based on statistical methods should be preferred. In this study we present the optimization of tannase production by *Aspergillus niger* GH1 in SSF utilizing a strategy based on statistical experimental designs.

Materials and methods

For this study *Aspergillus niger* GH1, previously isolated form creosote bush (*Larrea tridentata*) leaves was utilized. Fungal spores were stored at -50 °C in a cryo-protector system composed of glycerol and skim milk. Microorganism was propagated by transferring conserved spores to Erlenmeyer flasks with potato dextrose agar and incubating at 30 °C for 5-10 days. Inoculum was prepared adding 0.01 % (v/v) Tween 80 and scraping with a magnetic stirrer. Spores were counted in a Neubauer chamber before inoculation.

Tannase production was carried out in a SSF system using polyurethane foam (PUF) as inert carrier and a defined culture media with tannic acid as sole carbon source. Culture broth was inoculated with 1 x 10^7 spores per mL and 10.5 mL of inoculated medium were mixed with 4.5 g of pulverized PUF. Material was mixed and packed into a column bioreactor. Reactors were incubated at controlled temperature for 72 h and constant air flow rate. After incubation, crude extract was recovered by washing with acetate buffer (50 mM, pH 5.0) and compressing the fermented material. Crude extract was centrifuged and filtered through 0.45 µm nylon membrane.

Optimization of tannase production was performed in two stages. First, the effect of ten variables on tannase production was determined utilizing a Plackett-Burman experimental design. Then, the significant factors were optimized utilizing a Box-Behnken design.

Plackett-Burman experiment consisted of 12 different treatments. The independent variables included carbon source (tannic acid concentration, 1.25-5 %), the salts concentration (K_2HPO_4, 0.1-0.2 %; $NaNO_3$, 0.3-0.6 %; KCl, 0.05-0.1 %; $MgSO_4 \cdot 7H_2O$, 0.05-0.1% and $FeSO_4 \cdot 7H_2O$, 0.0024-0.0048 %), incubation temperature (30-40 °C), inoculum age (5-10 d), air flow rate (20-60 mL/min) and initial pH value (4.0-5.0). Dependent variable was extracellular tannase production, which was measured by the spectrophotometric method of the methanolic rhodanine (Sharma *et al*., 2000). This experiment was carried out by triplicate.

Factors that significantly affected tannase production were optimized by response surface methodology using a Box-Behnken statistical design. Three different variables at three levels in 12 different trials and 3 central points were evaluated. The experiment was carried out with four replicates.

Data obtained from Plackett-Burman and Box-Behnken designs were subjected to analysis of variance with Statistica 7.1 software (Statsoft, Tulsa, USA) to find the variables with statistically significant effect. Box-Behnken design data were also subjected to multiple regression analysis. Regression coefficients for lineal, quadratic and lineal interactions for each variable were adjusted to a polynomial equation of second order (Eq. 1).

$$y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 A^2 + \beta_5 B^2 + \beta_6 C^2 + \beta_7 AB + \beta_8 AC + \beta_9 BC \quad \ldots \quad \text{Eq. 1}$$

Where y = tannase activity (U/L); *A*, *B* and *C* are the coded levels of tannic acid, initial pH and incubation temperature, respectively; and βi are the regression coefficients of each variable. This mathematic model was optimized with the solver application of Microsoft Excel Office 2007 (Microsoft, USA).

The tannase production under optimal conditions was kinetically monitored for 72 h, at an interval of 8 h and analyzed for tannase activity, protein concentration and CO_2 concentration in the outlet air. This experiment was carried out with two replicates.

Results and discussion

Statistical analysis of data from the Plackett-Burman experiment indicated that only three (tannic acid concentration, initial pH and incubation temperature) out of the 10 independent variables evaluated significantly (95 % confidence level) affected the tannase production by *A. niger* GH1 in solid state fermentation (Figure 1).

It was observed in this study that none of the salts in the culture medium had a significant effect over tannase production. It might be due to the fact that the salt requirements of *Aspergillus* are relatively low. However it was also observed that high levels of salts utilized did not inhibit either growth or enzyme production by *A. niger* GH1, which indicated the salt tolerance of this strain.
A small decrease in tannase activity was recorded with an increase in the air flow rate, probably due to the humidity loss; however this effect was statistically not significant. Inoculum age too did not affect significantly tannase production, although earlier Kar *et al*. (1999) reported that age of inoculum is a key factor that affects tannase production.

The three variables that significantly affect the tannase production (tannic acid concentration, initial pH and incubation temperature) optimized using response surface methodology. For this aim, a Box-Behnken statistical design was employed. Analysis of variance confirmed the linear effect of tannic acid concentration, initial pH and incubation temperature on tannase production. The quadratic effects of tannic acid concentration and incubation temperature as well as an interaction between tannic acid concentration and pH were found to be statistically significant at 95 % confidence level (Figure 2).

Data obtained were analyzed by multiple regression and the following mathematic model was obtained (Eq. 2):

$$y = 459.68 + 880.46A - 995.71B - 564.96C + 923.01A^2 + 622.44B^2 + 898.88C^2 - 642.27AB + 154.18AC + 525.75BC \quad \ldots \text{(Eq 2)}$$

This model was optimized within the experimental range. Maximal theoretical value was reached at highest tannic acid concentration (50 g/L), lowest pH value (4.0) and at lowest temperature (30 °C). These tendencies can be observed in the response surface plot shown in Figure 3.

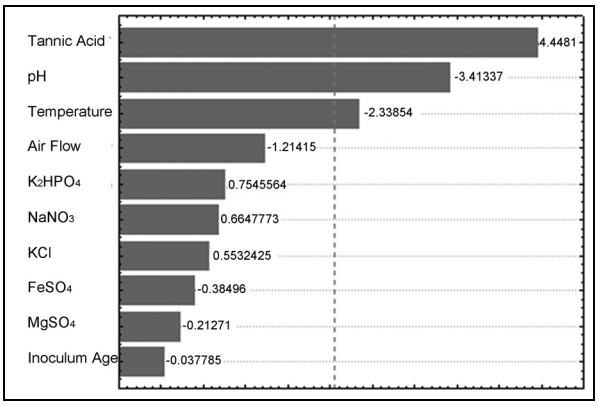

Figure 1. Standardized effects of 10 variables on tannase production according to Plackett-Burman statistical design.

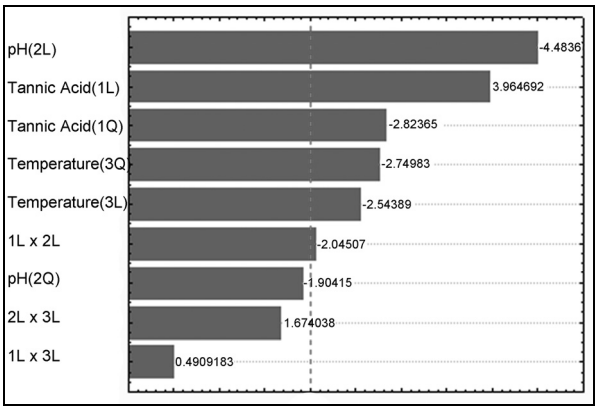

Figure 2. Standardized effects of tannic acid concentration, pH and incubation temperature on tannase production according to Box-Behnken statistical design.

Tannic acid concentration had a positive effect over tannase production, and is widely reported and well known as an inductor for tannase production. Although induction and repression mechanisms for tannase production are not clearly understood, it is well known that high concentration of tannic acid improve tannase production. In the present study, the highest tannase production was reached at 50 g/L of tannic acid as sole carbon source, and response surface plots suggest that still higher tannase production can be obtained if tannic acid concentration is increased (Figure 2).

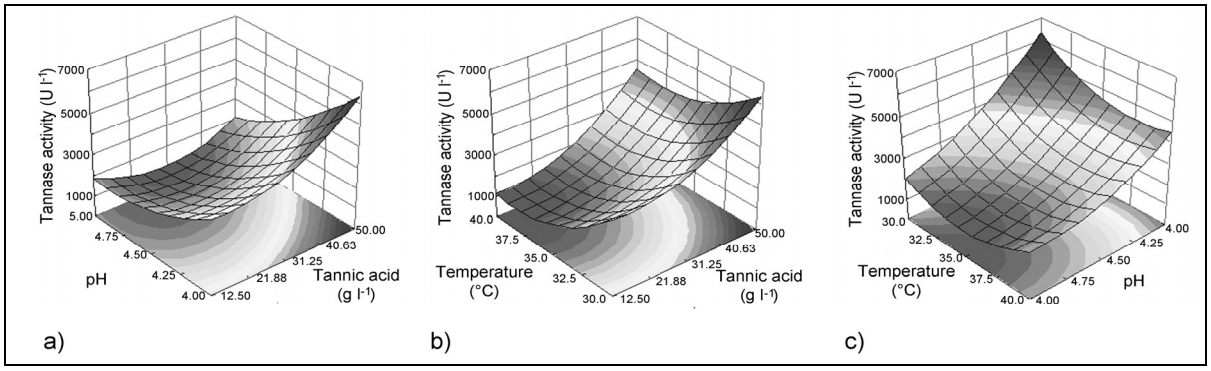

Figure 3. Response surface plot of tannase production as a function of: a) pH and tannic concentration at optimal temperature. b) Temperature and tannic acid concentration at optimal pH. c) Temperature and pH at optimal tannic acid concentration.

Increase of initial pH showed a negative effect on tannase production. In this study, optimum initial pH for the production of tannase by *A. niger* GH1 was found to be 4.0, while optimal pH for tannase production by *Aspergillus* spp. in SSF are found between 5.0 and 5.5 (Mukherjee and Benerjee, 2004; Manjit *et al*., 2008). Low initial pH value in optimized medium represented a technical advantage because it avoids the need for adjusting pH value with alkalis, reduce the time of preparation and the possibility of contamination.

Because of the origin of this fungal strain, it was hypothesized that it could need high temperature for their optimal growth. However, results shown that maximal tannase production was reached at relatively low growth temperature. Low temperature of incubation could be a technical advantage for industrial production due to low energy requirements. Decrease in tannase production at high temperature could be related to water loss due to evaporation or thermal denaturation rate of the enzyme.

Maximal tannase activity obtained under optimal conditions (7955 ± 798 U/L) was 1.97 times higher than the values obtained with the initial conditions (run 12 of Plackett-Burman design). In other studies Raaman *et al*. (2010) obtained a 1.23 fold increase in the extracellular tannase production by *Paecilomyces variotii* with a one factor at time method. Sharma and co-workers (2007) optimized tannase production by *A. niger* using response surface methodology and obtaining 2.01 fold improvement on enzyme production.

Conclusion

Tannase production by a wild strain of *A. niger* in SSF was increased by manipulating culture conditions. Using a two stage statistical strategy an increase in the enzyme activity by 1.97 times over that of initial growth conditions was obtained. The Plackett-Burman experiment allowed the identification the key factors that affected the tannase production. These factors were successfully optimized by response surface methodology. The highest tannase production was obtained at relatively low temperature (30 °C), a high concentration of tannic acid (50 g/L), and a unique low pH (4.0). Experimental strategy utilized in this work proved to be an efficient method for optimizing enzyme production in solid state fermentation.

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CFD studies on heating of canned food in aluminum based semi rigid containers

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Sterilization of food in cans has been well studied both experimentally and theoretically, but little work has been done on sterilization of food in aluminum based semi rigid packaging. In this study, transient temperature and the shape of the slowest heating zone (SHZ) have been established for a uniformly heated three dimensional Alupak packaging type B 232 containing a mixture of solid and fluid food, during all steps of sterilization, through modifications to container geometry by Gambit 2.3.16. The whole domain of three-dimensional can sitting in an upright position used in this simulation was divided into 498509 cells. Gambit software was used to create and modify mesh. The computational fluid dynamics (CFD) code Fluent 6.3.26 was used to establish shape of SHZ and governing equations for energy were solved numerically using a finite volume method. The model food was assumed to have constant properties. To validate the simulation, food containing meat, red beans and special sauce was sterilized in water cascading retort and variation of midpoint temperature according to the time in the can containing food was compared with predicted data from simulated model. The thermocouple used in validation was attached to Ellab data logger CTF9004 with PT100 cables. Data logger was connected to a personal computer and E-val software version 2.1 was used to export data. The experimental measured temperature at SHZ in the container was compared with that predicted by model. Both results were found to be in good agreement and the difference was not significant at $p<0.01$.

Simulation of transient natural convection in canned convection heated foods using computational fluid dynamics (CFD) approach

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In convection heated foods, process optimization studies is not common (in fact, a mathematical optimization study was not found) in the literature since the requirements for the solutions of energy, continuity and momentum equations results in a very long computing times. In addition, thermal buoyoncy effects leading to the natural convection should also be included in the solution.

Use of axi-symmetric approaches in vertical cans lead to 2 dimensional models resulting in rather short computation times. However, in horizontal cans including liquid products, this approach is not possible. Assuming to neglect the effect of natural convection in the horizontal direction compared to the vertical direction, it would be possible to use 2 dimensional approaches in the horizontal cans without applying the axi-symmetric approach. Therefore, the objective of this study was to simulate the heat transfer in horizontal cans of convection heated foods and validate the simulation results with experimental results to demonstrate the use of 2 dimensional approaches.

In the experimental part, the cans with model liquids (water and CMC – sodium carboxy-methyl cellulose) were prepared in 500 g capacity, and after mounting the thermocuples in the cans, thermal processing was carried out in an autoclave and the experimental results were compared with the simulation results for validation of the simulation models. In the simulation part, continuity, energy and momentum equations were solved using Ansys CFX.

The results validated the use of 2 dimensional approach in heat transfer modeling of liquid containing horizontal cans demonstrating the reduced computational times.

Aseptic processing of low-acid multiphase food products: Particle flow monitoring and process validation

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The need for a process that is alternative to traditional canning to produce low-acid multiphase food products is increasing, as the consumers become more demanding about the quality, variety, and convenience of the food they eat. Continuous flow processing (aseptic processing) in this regard has been the subject of considerable interest to many researchers. Application of aseptic processing to multiphase foods, however, has been hindered due to the challenge to prove that the least-treated particle has received an appropriate minimum thermal treatment. Therefore, there is a need for a simple, inexpensive, reliable methodology and tools for monitoring and validating aseptic processing of multiphase foods.

Thermo-magnetic switch implants were developed for non-invasive temperature determination of moving particles during continuous flow thermal processing of multiphase foods. These are miniature magnets coupled by their similar poles (magnetic field cancellation) using eutectic alloys. The alloy solder melts at the eutectic phase change temperature resulting in a detectable change in magnetic field strength due to the reorientation of magnets (magnetic field addition). The change in the magnetic field strength, and hence attainment of the pre-determined temperature level, can be detected by magneto-resistive sensors placed on the external surface of the tubes in the sterilization system.

By using the present methodology, processors can properly design their continuous thermal processes and increase the quality of their products. The advantages of aseptic processing can then be fully utilized.

Processing of freshly squeezed tomato juice by pulsed electric fields with respect to quality parameters

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Processing of freshly squeezed tomato juice by pulsed electric fields with measurement of important quality parameters were studied. Tomato juice samples were processed by 3 μs pulse duration, 30 kV/cm electric field strength and 400 pps frequency. While other processing parameters were constant, the total treatment time changed from 0 (control) to 1309 μs.

To determine whether PEF treatment causes any changes in the important quality parameters of tomato juice pH, °Brix, conductivity, color (L, a, b), chroma, hue, titratable acidity, total phenolic substances, total antioxidant activity of the tomato juice samples were measured. Moreover, FT-IR spectra, DSC spectra, particle size, zeta potential and viscosity of the samples in addition to lypoxigenase activity were also determined. It was detected that except for lypoxigenase activity no significant change was determined between the control and PEF treated samples ($p>0.05$). Inactivation of lypoxigenase increased with increased treatment time ($p\leq0.05$).

Based on the obtained results it can be concluded that PEF processing is an effective method to process tomato juice with significant reduction of lypoxigenase activity without significantly affecting important quality parameters. Thus, the effectiveness of PEF processing for tomato juice processing should be extended with determination of other quality parameters such as lycopene content and sensory properties in order to evaluate the potential of PEF for tomato juice processing.

Effect of pomegranate (*Punica granutum*) and rosemary (*Rosmarinus officinalis* L.) extracts on shelf-life for chilled Greenland halibut (*Reinhardtius hippoglossoides)* fillets in modified atmosphere packaging at 2 ºC

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Abstract

The present study evaluated the effect of pomegranate extract (1% v/w) and rosemary extract (1% v/w) as natural preservatives as well as their combination (1% v/w) on shelf life extension of previously frozen and chilled Greenland halibut fillets in modified atmosphere packaging (MAP, 40%CO_2/60%N_2) at 2 °C. Parameters that were monitored were: microbiological (aerobic plate counts (APC), lactic acid bacteria (LAB), *Lactobacillus* spp., and *Photobacterium phosphoreum*), biochemical (pH, thio-***barbituric acid*** (TBA), trimethylamine (TMA) and total-volatile-nitrogen (TVN)), and sensory (color, flavor and texture) attributes. For microbiological results, irrespective of treatments, APC reached levels $\geq 10^7$ CFU/g during storage. The spoilage microflora of fillets in MAP was dominated by LAB, but the concentration of *Lactobacillus* was very low. During storage, *P.phosphoreum* was not detected in any sample. Among the chemical indices examined, TBA values of control samples exceeded the limit of 2 mg malondialdehyde (MDA)/kg (2.78 and 4.08 mg MDA/kg) on days 18 and 23 of storage, respectively, while TBA values for samples treated with extracts remained below the limit throughout the storage period. Final (TMA) and (TVN) values for all treatments ranged between 0.15 to 0.37 mg N/100 g and 16.90 to 24.10 mg N/100 g after 23 days of storage, respectively, not exceeding upper acceptability limit set by EU. Sensory analysis correlated well with TMA and TVN analysis, indicating a shelf life of longer than 23 days for all samples. The research was supported by the Federation of European Microbiological Societies (FEMS) and performed during a research visit by İlke Uysal Ünalan at DTU Food.

Introduction

In recent years, there has been a growing interest in natural ingredients, especially of plant origin, due to their greater application in food industry for increasing consumer acceptability, palability, stability and shelf-life of food products and these ingredients has started to replace the synthetic preservatives. Compounds obtained from natural sources such as grains, oil seeds, spices, honey, fruits and vegetables have been investigated (1). Extracts from spices, rosemary, thyme, and sage are found to have antioxidant properties similar to or greater than BHA and BHT (2, 3). Pomegranate (*Punica granutum*) contains important bioactive compounds and has been widely consumed in many different cultures for thousands of years. Pomegranate is one of the important fruits grown in Turkey, Iran, USA, Middle East, Mediterranean and Arabic countries (4). Pomegranate fruit parts are rich in tannin and phenolic compounds and are known to possess considerable antioxidant effects (5) and antimicrobial activities (6, 7). (8) studied the effect of pomegranate sauce on the quality of marinated anchovy during refrigerated storage and found that the juice concentrate had a positive effect on the quality and shelf life of marinated fish. Another natural ingredient that could possess antioxidant activity is rosemary (*Rosmarinus officinalis* L.). In addition to inhibition of lipid oxidation, several authors have reported that some of the compounds present in rosemary extract/essential oil possess antibacterial properties (9, 10, 11). To the best of our knowledge, the antimicrobial and antioxidant effects of pomegranate and rosemary extracts on chilled Greenland halibut (*Reinhardtius hippoglossoides*) fillets have not previously been studied and reported. The objectives of this study were to evaluate the effect of pomegranate and rosemary extracts on changes in microbiological, physico-chemical and sensory quality attributes of MAP halibut stored at 2°C.

Materials and Methods

Materials Frozen

Greenland halibut fillets (*Reinhardtius hippoglossoides*) were obtained from Royal Greenland Seafood A/S, Aalborg, Denmark. 1% full extract of rosemary (*Rosmarinus officinalis*) was obtained from Food Quality Inc., Akureyri, Iceland.

Determination of total phenolic content

Freeze-dried ethanol pomegranate extracts were prepared by slight modification of the method given by (12). The total phenolic concentrations of extracts were determined using the Folin-Ciocalteu method described previously (13). Total phenolics of extracts were calculated from standard gallic acid solutions (0-0.5 mg/ml), and expressed as mg gallic acid equilavents (GAE) per g extract. The estimation of phenolic compounds in the extracts was carried out in triplicate and the results were averaged.

Preparation of fish samples

Frozen halibut fillet was stored at -40 °C and then thawed overnight at 8°C. Skinless and interleaved fillets prepared by a local fish processor were cut into pieces of appropriate size for packaging. 120 g fillet pieces for microbiological and chemical analysis and 100 g fillet pieces for sensory analysis were used. Fish samples were assigned to one of the following four treatments: Control (fish without any extract); P (pomegranate extract, 1% v/w); R (rosemary extract, 1% v/w); PR (pomegranate-rosemary extract combination (1% v/w)). After addition of extracts, samples placed in plastic bags were thoroughly tumbled. After treatment the fish fillets were kept in plastic bags for 1 h and then randomly placed in plastic trays and packed using a Multivac C500 packaging machine (Multivac Ltd., Vejle, Denmark). Fillets were packed in a modified atmosphere with 40% CO_2 and 60% N_2 (AGA, Copenhagen, Denmark). Packs of thawed halibut in MAP were stored at 2°C. The temperature of all sub-batches of halibut was followed during chilled storage by data loggers (Tinytag, Gemini Data Loggers Ltd., Chichester, UK).

Sampling and analyses

At each sampling time three packages were evaluated. The composition of the atmosphere in packs was determined by a gas analyser (Combi Check 9800-1, PBI Dansensor, Ringsted, Denmark), drip loss was measured as previously described (14) and sensory, microbiological and chemical analyses were carried out as indicated below.

Microbiological analyses

Each treatment of halibut fillets was analyzed for concentrations of aerobic plate counts (APC) and lactic acid bacteria (LAB) during chilled storage. Concentration of *P. phosphoreum* and *Lactobacillus* were determined on the first day and on the 18th day of chilled storage, and at the end of chilled storage, respectively. Twenty grams of skinless halibut flesh were diluted ten-fold in chilled physiological saline (PS) (0.85% NaCl and 0.1% Bacto-Peptone) and homogenized for 60 s in a Stomacher (Seward Laboratory Blender, London, UK). From this homogenate a series of ten-fold dilutions was prepared with chilled PS. APC were determined by spread plating on prechilled plates of Long and Hammer (LH) agar with 1% NaCl (15 °C, 7 days) (15). LAB were determined by pour plating in nitrate actidion polymyxin (NAP) agar with pH 6.2 (25 °C, 3 days) (16). *P. phosphoreum* was enumerated at 15°C by a conductance based incubation method using three vials for each halibut sample (17). *Lactobacillus spp*. was enumerated by spread plating on Rogosa Agar followed by incubation at 25°C for 2 days.

Physico-chemical analyses

To characterize halibut fillets, samples were homogenized using a blender and dry matter, lipid content, NaCl content, organic acids, pH, TMA, trimethylamine-N-oxide (TMAO) and TVN were determined in triplicate. These analyses were carried out prior to chilled storage and as previously described (14). The oxidative stability of halibut fish fillets were determined with the thiobarbituric acid-reactive substances (TBARS) assay as described by (18). ***TBA*** value was determined in triplicate. TBARS were calculated from a standard curve of MDA. TBA number was calculated as mg MDA per kg fish sample. During chilled storage of halibut pH, free fatty acids, concentration of TMAO, TVN, TMA and TBA value were determined.

Sensory analyses

At each sampling time two portions of cooked samples from each treatment of chilled halibut were evaluated by 3 trained people. Portions of 50 g were heated in coded porcelain bowls in a convection oven (100°C, 28 min) and then served to the panelists. In addition to halibut stored at 2 °C, freshly thawed halibut samples, previously kept at -40 °C, were included in each sensory evaluation as references. Color, flavour and texture of samples were evaluated by using a simple scale with three classes, where three corresponds to a spoiled sample (14). The shelf life of halibut at each treatment group was defined as the time when 50% or more of the panelists determined cooked samples to be in class three.

Statistical analysis

For the microbiological, chemical and physical analysis the effect of storage time and different treatments on chilled MAP halibut fillets were analyzed by one-way analysis of variance (ANOVA) method ($p<0.05$). Comparison of means was performed using Tukey's (HSD) test.

Results and Discussion

Total phenolic content

The freeze-dried ethanol pomegranate peel extract gave a yield of 21 mg g^{-1}. Pomegranate peel extract had high phenolic content of 481 mg GAE g^{-1} dry extract. 1% aqueous rosemary extract had 30.2 mg GAE g^{-1} dry extract (According the producer the solution was 1%). Fish fillets (100 g) contained 24 mg total phenolic content for pomegranate extract and 0.302 mg total phenolic content for rosemary extract as GA equivalent.

Microbiological changes

Fillets were stored at an average temperature of 2 °C with very little variation in the storage temperature. The initial concentration of APC of chilled halibut fillets in MAP was *ca* 10^3 CFU g^{-1} whereas counts on NAP corresponded to below or close to 10 CFU g^{-1}. During the storage period *P. phosphoreum* was not detected in any sample. Irrespective of treatments, APC reached levels $\geq 10^7$ CFU g^{-1} during storage. During chill storage no significant differences in APC ($P>0.05$) except storage of 10 day were observed in all four treatments. The spoilage microflora of fillets in MAP was dominated by LAB but the concentration of lactobacillus as determined on Rogosa agar was low (Table 1). The spoilage microflora was most likely dominated by *Carnobacterium* as these LAB are known to be resistant to frozen storage of fish and relatively resistant to the CO_2 concentrations in MAP. Absence of the the highly CO_2–resistant and Gram negative spoilage bacterium *P. phosphoreum* in the dominating microflora suggests it has been inactivated during the frozen storage of the studied fillets prior to thawing and chilled MAP storage (19, 20, 21).

Table 1. Microbiological changes of chilled MAP halibut fillets during chilled storage at 2 °C

Treatments	Storage Period (days)						
	0	**3**	**7**	**10**	**14**	**18**	**23**
Aerobic Plate Count							
A	3.10±0.07^{A}	3.34±0.34^{A}	4.76±0.70^{B}	5.14±0.18^{a,B}	6.64±1.24^{C}	7.55±0.03^{C}	9.37±0.04^{D}
B	3.51±0.60^{A}	3.34±0.37^{A}	5.04±0.24^{B}	6.12±0.17^{b,C}	7.07±0.21^{D}	7.75±0.34^{E}	9.46±0.01^{F}
C	3.31±0.38^{A}	3.57±0.08^{A}	5.25±0.30^{B}	6.40±0.06^{b,C}	7.74±0.07^{D}	7.76±0.11^{D}	9.38±0.14^{E}
D	2.97±0.28^{A}	3.51±0.44^{A}	5.26±0.17^{B}	5.98±0.46^{b,B}	7.18±0.14^{C}	7.93±0.28^{C}	9.50±0.05^{A}
Lactic Acid Bacteria							
A	1.00±0.00^{A}	1.36±1.23^{A}	3.98±1.43^{B}	4.17±0.18^{B}	5.18±1.49^{B}	6.48±0.06^{a,B}	9.09±0.23^{a,C}
B	0.68±1.18^{A}	1.10±0.95^{A}	3.26±0.03^{B}	4.85±0.40^{C}	5.64±0.31^{C}	7.06±0.19^{a,D}	9.26±0.08^{a,E}
C	1.85±0.47^{A}	1.97±0.03^{A}	3.39±0.58^{B}	5.59±0.26^{C}	6.14±0.67^{C}	5.53±0.37^{b,C}	8.88±0.24^{ab,D}
D	1.48±0.00^{A}	1.89±0.43^{A}	4.08±0.22 ^{B}	4.93±1.00^{B}	4.60±0.02^{B}	6.82±0.50^{a,C}	8.59± 0.13^{b,D}
Rogasa Agar, log (CFU g^{-1})							
A	-§	-	-	-	-	-	4.00±0.00
B	-	-	-	-	-	-	4.33±0.58
C	-	-	-	-	-	-	5.10±1.15
D	-	-	-	-	-	-	4.74±1.29

a-b : Different letters within each storage time denote significant difference at p<0.05.
A-F : Different letters within each treatment denote significant difference at p<0.05.
§ : Not measured

Physico-chemical changes

Salt content, water activity, dry matter and lipid content of halibut fillets between first and last chilled storage days changed insignificantly (P>0.05) between 0.24-0.34%, 0.994-0.996, 24-30%, and 13-16%, respectively (Table 2). Just after packaging the modified atmosphere contained CO_2 concentrations from 33.90 to 35.70%. At the 3rd day of storage CO_2 concentrations significantly decreased (P<0.05) to 21.17-22.33%. After 3rd day of storage CO_2 concentrations remained almost constant for all four treatments (Table 2). Drip loss of fresh and thawed fish is strongly species dependent with less than 2% drip loss for MAP salmon (20) and above 10-15% during chilled storage of thawed MAP cod (19). Drip loss is crucial because every loss in weight cause economical loss and affect consumer preference negatively. These losses were not significant (P >0.05) between consecutive days; however, they were significant when the initial (*ca* 5%) and final sampling dates (*ca* 12%) were compared but the inclusion of drip pads helps to reduce the problem. Glycerides, glycolipids and phospholipids are hydrolyzed by lipases to free fatty acids, which then undergo further oxidation to produce low molecular compounds, such as aldehydes and ketones. These compounds are responsible for off-flavour and off-odour and taste of fish. The initial FFA content in the chilled MAP halibut sample was 3.3±0.25%. At the end of storage average FFA value of samples was 5.94±0.74 % with no significant difference (P>0.05) between the four treatments during chill storage (Table 2). The release of FFA significantly increased from the initial value to the final value (P<0.05) during the storage period. Since the release of FFA content increased with time as found in this study (Table 2),

Table 2 Physico-chemical changes of chilled MAP halibut fillets during chilled storage at 2 °C

	Storage Period (days)						
	0	3	7	10	14	18	23
Dry matter, %							
A	28.72 ± 1.23	-§	24.60 ± 4.46	-	-	-	28.82 ± 3.02
B	28.72 ± 1.23	-	28.10 ± 2.32	-	-	-	29.87 ± 2.17
C	28.72 ± 1.23	-	29.65 ± 1.02	-	-	-	27.31 ± 2.68
D	28.72 ± 1.23	-	27.35 ± 1.37	-	-	-	28.83 ± 1.56
Water activity							
A	0.996 ± 0.002	-§	-	-	-	-	0.995 ± 0.002
B	0.996 ± 0.002	-	-	-	-	-	0.994 ± 0.004
C	0.996 ± 0.002	-	-	-	-	-	0.994 ± 0.002
D	0.996 ± 0.002	-	-	-	-	-	0.996 ± 0.002
Salt, *%*							
A	0.28 ± 0.02	-§	-	-	-	-	0.24 ± 0.06
B	0.28 ± 0.02	-	-	-	-	-	0.26 ± 0.04
C	0.28 ± 0.02	-	-	-	-	-	0.34 ± 0.01
D	0.28 ± 0.02	-	-	-	-	-	0.33 ± 0.04
Lipid, *%*							
A	16.88 ± 3.95^{A}	-§	14.23 ± 8.61^{A}	-	-	-	13.24 ± 0.52^{A}
B	16.88 ± 3.95^{A}	-	10.14 ± 2.42^{A}	-	-	-	12.64 ± 0.73^{A}
C	16.88 ± 3.95^{A}	-	13.74 ± 3.17^{A}	-	-	-	11.39 ± 2.06^{A}
D	16.88 ± 3.95^{A}	-	9.08 ± 0.63^{B}	-	-	-	12.60 ± 0.98^{AB}
Lactic acid, ppm							
A	312.0±173.49	-§	262.7±16.26	-	-	-	450.0±148.36
B	312.0±173.49	-	306.3±364.33	-	-	-	165.4±94.03
C	312.0±173.49	-	407.0±132.02	-	-	-	534.7±129.57
D	312.0±173.49	-	197.7±29.70	-	-	-	414.0±95.45
CO_2, (%)							
A	34.03 ± 0.76^{A}	21.70 ± 1.78^{B}	$21.40 \pm 1.41^{a,B}$	20.50 ± 0.26^{B}	20.97 ± 3.27^{B}	21.93 ± 1.23^{B}	$22.63 \pm 0.80^{a,B}$
B	33.90 ± 0.75^{A}	$21,17 \pm 3.47^{B}$	$20.23 \pm 0.81^{a,B}$	20.73 ± 1.24^{B}	22.13 ± 2.93^{B}	22.20 ± 5.69^{B}	$23.73 \pm 0.90^{a,B}$
C	35.70 ± 0.90^{A}	22.33 ± 0.35^{B}	$25.20 \pm 3.44^{b,B}$	23.10 ± 1.57^{B}	24.53 ± 4.62^{B}	21.20 ± 6.39^{B}	$19.20 \pm 1.18^{b,B}$
D	35.30 ± 0.53^{A}	21.67 ± 1.03^{B}	$20.50 \pm 0.46^{a,B}$	21.53 ± 0.95^{B}	21.37 ± 0.32^{B}	21.90 ± 0.92^{B}	$22.70 \pm 1.87^{a,A}$
Drip loss, % (w/w)							
A	4.71 ± 2.28^{A}	8.72 ± 1.50^{AB}	9.95 ± 2.72^{B}	12.38 ± 1.42^{B}	9.89 ± 2.10^{B}	9.58 ± 1.75^{B}	11.85 ± 2.56^{B}
B	3.39 ± 0.29^{A}	9.80 ± 3.17^{B}	10.52 ± 0.90^{B}	10.01 ± 1.84^{B}	10.57 ± 1.39^{B}	12.91 ± 2.71^{B}	11.90 ± 1.74^{B}
C	5.23 ± 1.14^{A}	7.98 ± 2.90^{AB}	10.26 ± 2.83^{AB}	11.85 ± 4.17^{AB}	14.05 ± 2.89^{B}	11.86 ± 2.9^{AB}	11.49 ± 1.41^{AB}
D	5.00 ± 1.63^{A}	7.75 ± 2.32^{B}	8.94 ± 1.36^{AB}	11.85 ± 4.17^{B}	12.30 ± 0.56^{B}	11.81 ± 0.91^{B}	11.52 ± 3.93^{B}
Trimethylamine-N-oxide (TMAO), mg-N 100 g^{-1}							
A	73.15 ± 8.45	-§	-	-	-	-	68.23 ± 16.18
B	73.15 ± 8.45	-	-	-	-	-	63.39 ± 14.35
C	73.15 ± 8.45	-	-	-	-	-	59.76 ± 7.66
D	73.15 ± 8.45	-	-	-	-	-	52.98 ± 7.12
Trimethylamine (TMA), mg-N 100 g^{-1}							
A	-3.37 ± 0.90	-§	-	-	-	-	0.15 ± 0.46
B	-3.37 ± 0.90	-	-	-	-	-	0.33 ± 1.34
C	-3.37 ± 0.90	-	-	-	-	-	0.37 ± 2.03
D	-3.37 ± 0.90	-	-	-	-	-	0.21 ± 0.33
Free Fatty Acid, *%*							
A	3.33 ± 0.25^{A}	-$^{\delta}$	5.03 ± 2.50^{B}	-	-	-	6.36 ± 1.59^{B}
B	3.33 ± 0.25^{A}	-	6.66 ± 0.65^{B}	-	-	-	4.89 ± 0.96^{AB}
C	3.33 ± 0.25^{A}	-	4.61 ± 1.82^{B}	-	-	-	5.95 ± 0.84^{B}
D	3.33 ± 0.25^{A}	-	6.79 ± 0.41^{B}	-	-	-	6.54 ± 1.75^{B}

A-B : Different letters within each treatment denote significant difference at $p<0.05$.
§ : Not measured

it is reported that there is a relationship between FFA release and loss of freshness (22). The average pH value was 6.9±0.1 with a small reduction in pH recorded throughout the 23 days of storage (data not shown). Other chemical changes included the formation of lactic, acetic and citric acid. No significant difference ($P>0.05$) between all four treatments in the concentration of lactic acid was found during storage (Table 2). Acetic acid formation was initially below to detection limit and at the end of storage the

values reached 128 ppm but citric acid formation was below to detection limit during storage period (data not shown). The most common instrumental indices for assessing fish spoilage are TVN and TMA (23). 5-10 mg TMA 100 g^{-1} sample has been reported as the limit for sensory acceptability of fish (24). Although TMA values for the four treatments significantly increased ($P<0.05$) during the storage these value in all treatments were below limit level at the end of storage. Levels of 25 to 35 mg TVN 100 g^{-1} fish are regarded by the European Union as limits for sensory acceptability of fish. Initial concentration of TVN was 7.1±1.36 mg-N TVN 100 g^{-1}. No significant effect ($P>0.05$) of treatments in TVN formation was observed during the storage at 2 °C. Significantly higher TVN formation was only observed for all samples on day 23 at 2 °C. During storage TVN concentrations increased to 16.9-24.1 mg-N TVN 100 g^{-1} not exceeding upper acceptability limit set by EU (Figure 1). TBARS is one of the most widely used methods for measuring oxidative rancidity in food. The effects of addition of pomegranate and/or rosemary extracts on the oxidative stability of chilled MAP halibut fillets were shown in Figure 2. The TBA value significantly increased ($P<0.05$) in control samples throughout storage, however in treated samples the values increased insignificantly ($P>0.05$). Moreover, no difference was observed ($P>0.05$) between pomegranate and/or rosemary treated samples during the storage period and values in treated samples remained below or close to limit level (0.2 mg MDA kg^{-1} sample).

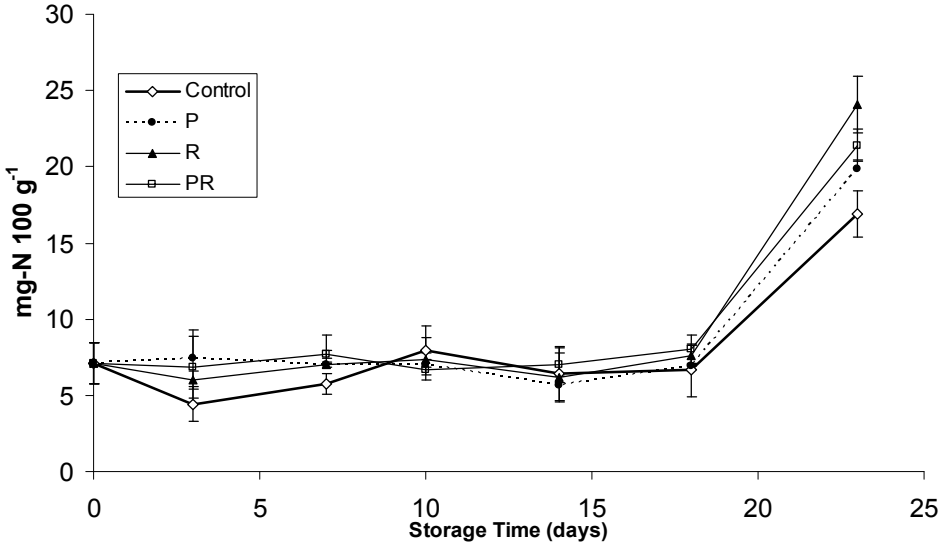

Figure 1. Changes in concentration of TVN of MAP halibut during chilled storage at 2 °C

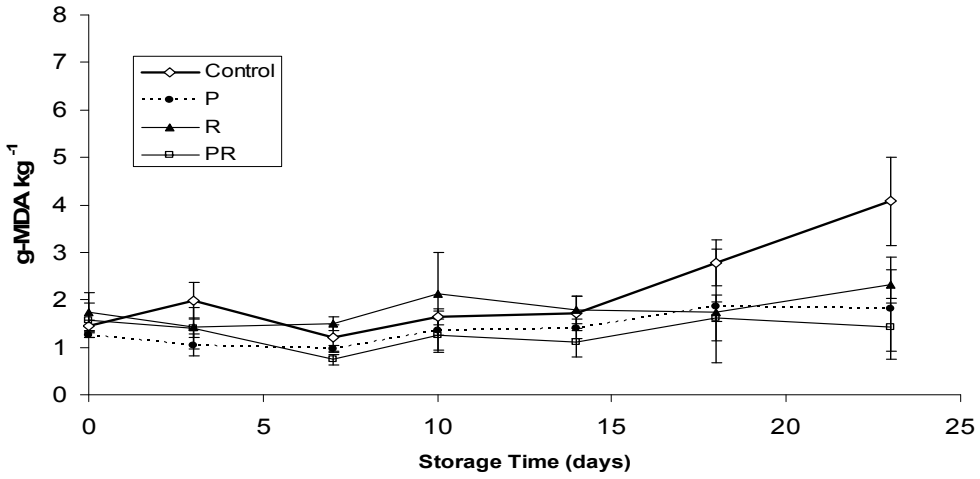

Figure 2. Changes in TBA values of MAP halibut during chilled storage at 2 °C

Sensory changes and shelf-life

The sensory spoilage characteristics of chilled MAP halibut were similar during storage and results for all treatments are shown Table 3. Dry texture and sour taste was the main sensory spoilage characteristics determined at all treatments. Particularly, the yellow colour of pomegranate extract-treated halibut samples (group P and PR) differed from other samples. In fact, at the first sensory analyses halibut fillets in group P and PR became light yellow, differed markedly from reference sample and samples in group Control and R. Difference between smell of halibut samples treated with or without extracts was not differentiated by panelists during the storage. For all treatments the sensory shelf life of chilled MAP Greenland halibut fillets was observed as longer than 23 days at 2 °C.

Table 3 Sensorial changes of chilled MAP halibut fillets during chilled storage at 2 °C

	Type of Treatments				
	Reference	A	B	C	D
Shelf-life (d)	>23	>23	>23	>23	>23
Sensory spoilage characteristics	White surface colour; good flavour; soft texture	White surface colour; sour flavour; chewy and dry texture	Yellow surface colour; sour flavour; dry texture	White surface colour; sour flavour; dry texture	Yellow surface and edges colour; sour flavour; chewy and dry texture

Conclusion

Sensory analysis correlated well with TMA and TVB-N analysis, indicating a shelf life of longer than 23 days for all samples. Despite no significant antimicrobial pomegranate and rosemary extracts, results provides first insight into their antioxidant effect on chilled halibut fillets in MAP and opens new frames for further investigations. From a microbiological point of view, the combined use of the tested natural preservatives and a packaging system with a 40% CO2, had no beneficial effect on microbial spoilage of the studied halibut fillets.

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The relationship between the color variables and moisture content of tomato purees during convectional drying

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Abstract

Tomato purees were dried at 100 ^{o}C by conventional heating. The moisture removal from tomato purees was recorded at regular time interval (60 min.) and color variables as Hunter L, a, b, chroma, hue angle and total color difference (ΔE) values were estimated. The changes in color variables were simulated using exponentional, polynomial, logaritmic, power and rational models. The exponential and power relationships were found among the color variables of tomato purees and moisture content due to statistical results. Surface curve fittings were applied due to changes in color variables and non-lineer regression analysis was used to determine the model coefficients. A close relationship was found among the color variables and moisture content of tomato purees.

Key Words: Hunter variables, tomato purees, moisture content, exponential and power model

Introduction

Tomato (Lycopersicon esculentum) commonly described as a vegetable food and adapted to various uses in the fresh form in salads, purees form in meals or soups for diet of diverse cultures in the world. It is grown worldwide on a variety of soils due to climatic conditions. United States, Turkey, Italy and Spain are the main tomato growing countries. Tomatoes contain a major content of antioxidant lycopene which reduces risk of cancer and cardiovascular diseases. The antioxidant activity of tomato extracts varies with the tomato variety (Guil-Guerrero & Rebolloso-Fuentes 2009). Lycopene pigment contributes to its red colour and helps in lowering DNA damage as an antioxidant (Abu-Jdayil et al., 2004). The lycopene concentration varies 6.0-15.0 mg/100 g for the fresh tomato fruits and it's amount affected by a number of factors such as high temperature, long processing time, light, oxygen and acids (Akanbi & Oludemi 2004).

Color is spread throghout nature in food materials especially fruit and vegetables. Human beings observed the color due to perception of the wavelengths from the surface of the materials. Light strikes the food materials and it is reflected, absorbed or transmitted. The reflected amount refers the color of material depending on the amount of light and it's source, angle of view and etc. The color of the fruit and vegetables are related with the pigments. Red color of the tomatoes are related with to the lycopene pigments, carotenoids in tomatoes. Loss of lycopene and color degradation are affected by factors such as light, long processing time, high processing temperature, genetics, soil, plant nutrition, majurity and seasonal variations (Akanbi & Oludemi 2004;Dutta et al., 2007). Lycopene is related with to the color variables such as lightness, hue angle, hunter a*, hunter a*/b* and its content (Perkinz et al., 2006). Hue angle is the color value which defined as starting at + a axis that is expressed in degrees: zero (0^{o}, red), 180 ^{o} (green) and 270 ^{o} (blue) (Özkan et al. 2003). Visual color is an important factor that affects the acceptability of the product and plays an important role in appearance and processing of the food materials. The browning reaction such as Maillard reaction, pigment degradation, enzymatic browning and ascorbic acid oxidation occur due to thermal processing of food materials (Assawarachan & Noomhorn 2010). Thermal application can cause the changes in color parameters such as L, a*,b* and total color difference, hue angle and chroma (saturation index) that are widely used to describe color change during the termal processing such as strawberry juice and tomato puree (Rodrigo et al.,2007), spinach (Dadali et al.,2007), kiwifruits (Maskan 2001), pomegranate juice concentrate (Maskan 2006), grape juice and leather (pestil) (Maskan et al., 2002). Hunter parameters are used for describing visual colors (L, whiteness/darkness; a, redness/greeness; b, yellowness/bluness) and provide useful information

for the quality controls of fruits, vegetables and its products (Dadali et al., 2007). Total color change ΔE, chroma (saturation index) and hue angle are used to reveal color in food especially for green food materials and meats in addition to L, a* and b* values. Browning index refers to pureness of brown color and is important factor for drying process due to enzymatic and non-enzymatic browning reactions (Barreiro et al., 1997;Maskan 2001).

There is a close relationship between moisture content of food materials and color variables. It is reported that increase in moisture content caused an increase in Hunter L, b, chroma and hue angle values but decrease in a values of dried apricot (Özkan et al. 2003). Effect of moisture content on the color variables of walnut cultivars is significant and there were a decrease in L and an increase in a values of the walnut cultivars as the moisture content was increased (Altuntas & Erkol 2009). The color variables of soybean and soy-bulgur varied according to the moisture content. It is reported that there was a relationship among the color variables and color parameters of soybean (Bayram et al. 2004). The L and b values of persimmons were decreased during the drying procedure while a values were increased (Akyıldız et el. 2008).

The objective of this work was to examine the relationship between the moisture content and color variables of tomato purees during the thermal treatment.

Materials and methods

Materials

Fresh tomatoes (Lycopersicum esculentum) were purchased from a local market in Osmaniye, Turkey. Tomatoes were washed and stored at 4 ^{o}C at refrigerator.

Methods

Drying process and colour analysis

Tomatoes were washed in fresh water and grated. Tomatoes were crushed and sieved for the seeds and fibres. Drying process of tomato purees were conducted at drying temperatures (100 ^{o}C). Moisture loss was recorded at 60 min. time intervals during the drying process within an accuracy of 0.1 g. Color parameters were determined by using Hunter colorimeter with the scale of L, a and b at each 60 min. time intervals. Tomato samples were placed into the standard glass container and read at opponent color scales: L (100=bright, 0=dark), a (positive=red, negative=green) and b (positive=yellow, negative=blue). Three measurements were done for tomato samples and instrument was calibrated against a standart reference tile. The total color difference ΔE, chroma (saturation index), and hue angle were calculated due to the equations 1-3.

$\Delta E = \{(L_o-L)^2 + (a_o-a)^2 + (b_o-b)2\}^{1/2}$ (Franchis & Clysdale 1975) (1)

$\text{Chroma} = \{a_2 + b_2\}^{1/2}$ (Abers & Wrolstad 1979) (2)

$\text{Hue angle} = \tan^{-1}(b/a)$ (Anon 1976) (3)

where $L_{o,}$, a_o and b_o refer to color parameters for fresh samples; L,a and b refer the color parameters of the treated samples.

Surface curve fittings

Moisture loss from the tomato purees and color variables were plotted with three dimension, drying time (minutes), moisture content (%) and Hunter color variables by Matlab programmes (R2009b). The fitting procedure was carried out due to different mathematical equations such as polynomial, rational, cubic, power, exponential and logaritmic models. The relationship was examined among the color variables and moisture content according to the higher regression coefficient, root of mean square of error (% rmse) and

sum of square of errors (sse). For the numerical calculations, the software package programme of Matlab (R200b), the sum of square error (sse) (Doymaz 2007; Arifoglu 2005; Dadalı et al., 2007), regression constant (r^2) (Arifoglu 2005;Vega-Galvez et al., 2010) and the root of mean square error (% rmse) (St George et al., 2004; Mok & Hettarachchcy 1990) were used for curve fitting procedures.

Results and discussions

The moisture content of tomato purees was found as 94.58 % at the end of drying process. As seen in Figure 1, it can be observed that the moisture content of tomato purees increases with drying time at a constant rate and there is no constant rate period and drying process took place in the falling rate period. The moisture removal from the product was higher at drying temperature and it is similar for coconut pres cake (Jena & Das 2007), olive cake (Akgün & Doymaz 2005) and pomace of olive oil (Gögüş & Maskan 2006).

L value decreased with drying time (Figure 1) indicating that tomato samples become darker. L values changed between 36.59 and 25.37 during drying process. The a values of the samples changed between 38.09 and 25.83 . The positive a value indicates that the samples are more redness at first and samples lost their redness and become darker when dried. The changes in a values can be explained by the decomposition of the pigments and formation of brown pigments during the drying (Rhim et al., 1989; Maskan 2001). The b values of the tomato samples (Figure 1) decreased from 18.17 to the 15.75 for drying process. There were an increase in L, a, b, chroma but decreased in hue angle and ΔE during the drying of tomato purees. It is reported that there is a close relationship between L values and pigment degradations (Maskan et al., 2002; Ahmed et al., 2004). Decrease in L values was due to loss of red color due to the pigment destruction (Rhim et al., 1989). The total color difference ΔE, hue angle, chroma were calculated by using the equations (1-3) and estimated values were presented in Fig. 2. Total color differences of samples increased during drying process of tomato purees. The hue angles of tomato samples (Figure 2) were increased from 25.5 to 31.74 during thermal treatment of tomato purees. Chroma values indicating color saturation of tomato samples (Figure 2) decreased during drying process. An exponential relationship was found among color variables of L, b, ΔE, moisture content and power relationship was found among a, chroma, hue angles of tomato purees.

Exponential and power equations were given due to regression analysis (equation 4-5); and equation constants, regression coefficients, % rmse and sse values were presented in Table 1 according to the surface curve fittings.

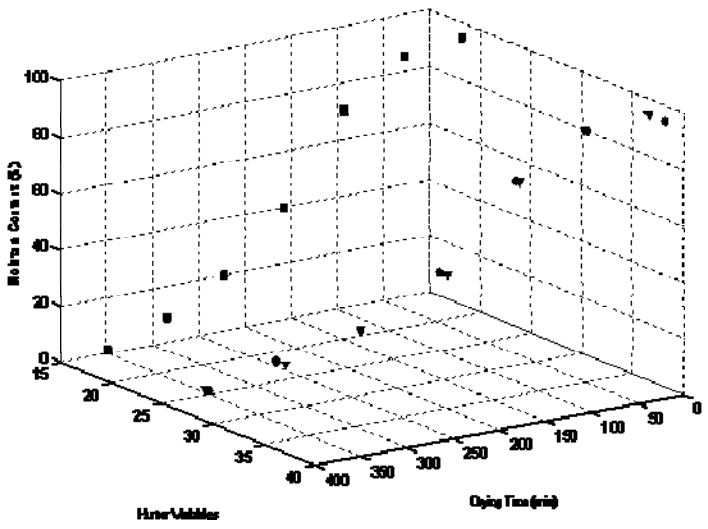

Figure 1. Surface curve fitting of tomato purees for Hunter L (●), a (▼), b (■) vs moisture (%) and time (min).

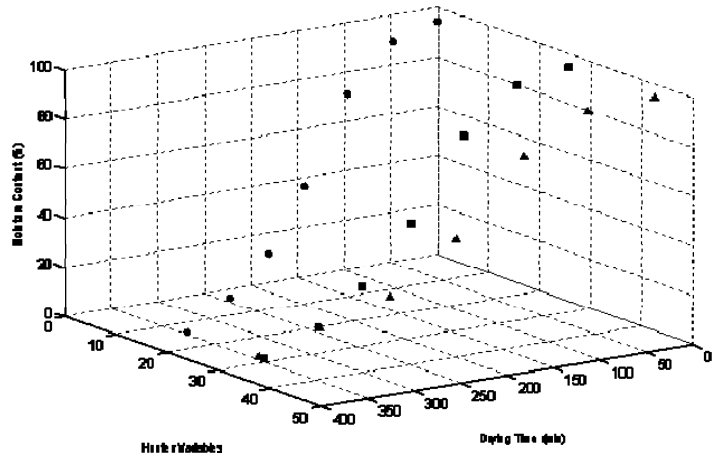

Figure 2. Surface curve fitting of tomato purees for Chroma (▲), Hue angle (■), ΔE (●) vs moisture (%) and time (min).

$$y = a\, e^{bx} + c\, e^{dx} \quad (4)$$

$$y = a\, x^{b} + c \quad (5)$$

Table 1. The equation constants, regression coefficients, rmse and sse values of curve fittings for tomato purees

Interactions	Constants				Regression Coefficients		
	a	b	c	d	r^2	rmse	sse
Hunter L-moisture	32.12	0.00129	-7.662	-0.0424	0.9941	0.4539	0.6182
Hunter b-moisture	-1.68 e^5	-0.00412	1.67 e^5	-0.00382	0.9752	8.524	21.80
ΔE-moisture	296.5	-0.2354	-202	-0.3987	0.9973	2.835	24.11
Hue angle-moisture	6.66 e^9	-5.447	-42.08	-	0.9342	12.01	57.74
Chroma-moisture	0.2026	1.869	-119.3	-	0.9699	8.12	26.38
Hunter a-moisture	0.1533	1.957	-88.4	-	0.9668	8.535	29.14

As seen in Table 1, among the color variables, drying time and moisture contents of tomato purees; regression coefficients were higher and % rmse and sse values were lower due to curve fittings. Regression coefficients r^2, were changed from 0.9342 to 0.9973. It means that there is a close relationship among the color variables and moisture content of tomato purees.

Conclusion

In present study, the color parameters of tomato purees L, a, b, hue angle, chroma and ΔE were used to investigate the changes in color of samples. Polynomial equation had a higher regression values according to the regression equation of color parameters among the equations. Increase in moisture removal from the samples, color variables of L, a, b, chroma decreased; hue angle and ΔE increased. There was a close relationship among the moisture content and color variables of tomato purees in terms of polynommial and exponential manner according to the statistical results.

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Supercritical extrusion (SCFX) in dough processing

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Abstract

Yeast-leavening is a semi-continuous process that requires constant temperature and humidity. Leavening by using supercritical carbon dioxide can eliminate the fermentation stage, thus reduce processing time to a great extent. In the present study, a dough formulation with 50 kg moisture/100 kg was leavened by adding 1.5 kg supercritical carbon dioxide/100 kg in a twin screw extruder at 37^0C. SCFX-leavened dough had a dough density of 420 – 430 kg/m^3 which was in agreement with dough densities reported for yeast-leavened similar dough. Apparent viscosity of SCFX-leavened dough was measured by using an on-line slit rheometer, and was found to vary from 37 – 23 Pa-s.

Introduction

Although bread-making is an ancient technology, demand for consistent texture, longer shelf life and narrow profit margins typical of the food industry are the major incentives for process control and improvements in technology. The bottlenecks of bread-making can be summarized as; the requirement for specific cultures that retain their activity under dried and frozen conditions (Cauvain, 1998) and an even distribution of gas bubbles to eliminate loaf defects (Shah et. al., 1998). Thus, final proofing involves an optimization between maximization of the loaf volume and minimization of the loaf defects (Hayman et. al., 1998). Dough rheology is related with dough development, thus is readily used for monitoring the bread-making process. For this purpose, empirical tests such as farinograhy, mixograhy, alveography, extensiography and viscoamylography (Faubion, 1986), and fundamental tests such as creep, compression, tension and flow behavior (Battacharya, 1993; Cuq et. al., 2002; Bagley et. al., 1998; Sharma et. al., 1993a; Sharma et. al., 1993b; Wang and Sun, 2002) are used, but there is very limited information on leavened dough rheology. Supercritical fluid extrusion (SCFX) is able to impart uniform size bubbles by allowing injection of supercritical carbon dioxide into the extrudate at low temperatures (Alavi et. al., 1999). Supercritical carbon dioxide solubilized in the dough system at high pressure diffuses out at the die acting as the expansion agent (Rizvi and Mulvaney, 1993). Use of SCFX allows a continuous bread-making process in which ready-to-bake leavened dough can be obtained where yeast is replaced by supercritical carbon dioxide as the leavening agent. The aim of the present study was to obtain SCFX-leavened dough having a comparable density with the conventional yeast-leavened dough. SCFX-leavening process also allowed on-line comparison of the rheological characteristics of non-leavened control dough and SCFX-leavened dough with constant processing history.

Materials and methods

Dry ingredient formulation consisted of 100 kg high-spring dominator flour, 6 kg sucrose, 3 kg whey protein concentrate, 1.5 kg salt, 0.25 kg DATEM, 0.25 kg locust bean gum and 0.25 g xanthan gum and $2.72\text{x}10^{-3}$ kg ascorbic acid. Feed rate of the dry mix was kept at $9.7\text{x}10^{-3}$ kg/s. Water ($4.85\text{x}10^{-3}$ kg/s), bakery fat ($2.43\text{x}10^{-4}$ kg/s) and supercritical carbon dioxide ($1.46\text{x}10^{-4}$ kg/s) were added.

Bread dough was extruded through the slit die of a self-wiping, co-rotating twin-screw extruder (Wenger TX-52 Magnum) with L/D=28.5:1, having 4.5 heads and equipped with supercritical injection ports located at L/D=24. The product temperature was kept between 36 – 38^0C, and extrusion pressure range was 14 - 15 MPa. Mean specific mechanical energy input was 260 kJ/kg. Continuous density measurement was carried out by weighing dough samples collected in a container with known volume. Density results reported were the arithmetic mean of five replicates for each extrusion run and five

extrusion runs for non-leavened control dough and the SCFX-dough (coefficient of variation=3 – 5 %). A slit rheometer with three ports from the die entrance at (Fig.1) was used for rheological characterization. Pressure was recorded automatically every 10 s by means of calibrated pressure transducers inserted at the second and third ports. Different shear rates based on different mass flow rates, and thus constant history were obtained by using the by pass valve situated at the first port. Mass flow rates through the die were measured every 30 s by weighing the material throughput at steady extrusion conditions. Data were collected in triplicate. Four extrusion runs were performed for the non-leavened control dough and the SCFX-leavened dough.

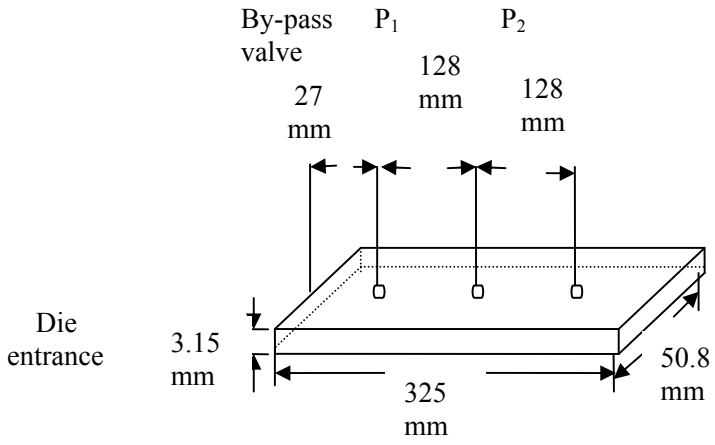


Figure 1. The slit die specifications

Shear stress at the wall was calculated from:

$$\tau_w = \frac{H}{2(\Delta L)}(\Delta P) \tag{1}$$

where ΔP (Pa) is the pressure drop measured at the transducers, H is the slit height and L is the distance between the two transducers. Apparent shear rate was obtained as:

$$\dot{\gamma}_{app} = \frac{6}{WH^2}\frac{\dot{m}}{\rho} \tag{2}$$

where m (kg/s) is the measured material throughput, W (m) is the slit width and H (m) is the slit height. Error calculated for each shear stress vs. shear rate combination was between ∓5 – 10 %. Rheological parameters were calculated from linear regression of log (shear stress) vs. log (shear rate) data using the power law model:

$$\tau_w = K'\left(\dot{\gamma}_{app}\right)^n \tag{3}$$

Where K´ (Pa-s^n) is the consistency index and n (dimensionless) is the flow behavior index. Wall shear rate was calculated as:

$$\dot{\gamma}_w = \frac{3n+1}{4n}\dot{\gamma}_{app} \tag{4}$$

Then apparent viscosity was calculated as:

$$\eta_{app} = K\left(\dot{\gamma}_w\right)^{n-1} \text{ where } \mathrm{K} = \mathrm{K}'\left(\frac{4\mathrm{n}}{3\mathrm{n}+1}\right)^n \tag{5}$$

Results and discussion

SCFX-leavened dough contained locust bean gum, xanthan gum and bakery fat unlike the conventional dough (Stear, 1990), besides relatively higher moisture and ascorbic acid contents. Higher moisture was necessary to promote dissolution of supercritical carbon dioxide. Ascorbic acid, known to strengthen the dough structure by disulphide bonds (Pomeranz, 1987) was crucial. This is because SCFX-dough does not contain an active leavening agent. Therefore, dough strength is necessary to prevent carbon dioxide loss following nucleation and bubble growth at the die exit. Gums were added to increase water absorption (Rosell et. al., 2001). Bakery fat was used to stabilize the membrane around the gas nuclei (Pomeranz, 1987), thus increasing the gas holding capacity. Besides, bakery fat protected the gluten – starch matrix from adverse affects of shear by providing lubrication.

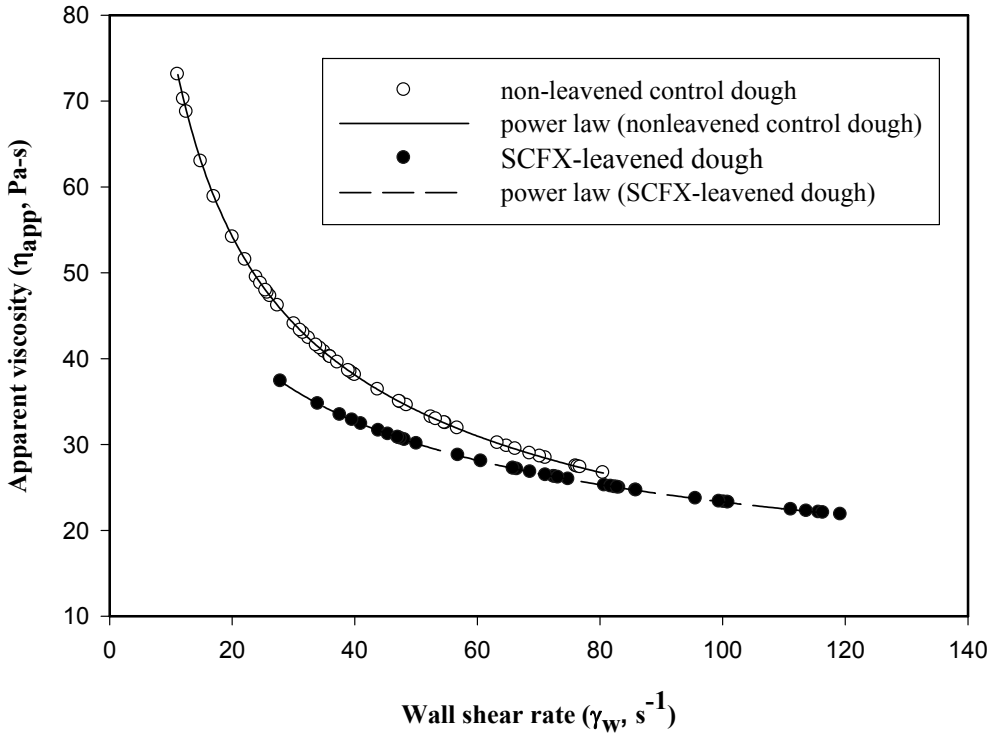
Dough density was taken as a measure of dough expansion. SCFX-leavened dough density was between 420 – 430 kg/m^3, similar to that of conventional leavened dough obtained upon proofing at 37^{0}C (Campbell et. al., 2001).

Table 1 Comparison of on-line measured rheological parameters with previous studies

Type of mixture	moisture (kg/ 100 kg)	Temperature (ºC)	Method	Type of dough	K (Pa-s^n)	n (dimensionless)	R^2
Bread mix	50	36 - 38	On-line slit die	Non-leavened control	250	0.49	0.8
				SCFX-leavened	128	0.63	0.9
Flour-water (Battacharya, 1993)	50	Not reported	Off-line slit die	Non-leavened	1309-1390	0.46-0.48	>0.9
Flour-water (Sharma et. al., 1993a)	64.5	21	Off-line capillary die	Non-leavened	1615-3115	0.24-0.46	>0.9
Flour-water (Cuq, et. al., 2002)	45	30	Off-line capillary die	Non-leavened	1090-405	0.45-0.63	>0.9

The usual practice is basing the rheological properties of leavened dough on the rheological properties of non-leavened dough (Faubion, 1986). These inferences may be fallacious due to the fact that leavening results in a decrease in pH, emerging gas bubbles and differing dough history due to mixing, forming and proofing may all change dough rheology. However, it has not been possible to perform fundamental tests on leavened dough due to the fact that these tests involve shear, elongation or compression which in turn will disturb the gas bubble distribution and will end in release of the gas bubbles. On-line rheological data of leavened dough at constant history could be obtained by application of the SCFX technology as SCFX-leavened dough was extruded through the slit die designed to perform as a slit rheometer. Regression results (Table 1) of shear stress vs. shear rate data gave flow behavior indices of 0.49 for the non-leavened control and 0.63 for the SCFX-leavened dough characterizing the classical shear-thinning behavior of dough (Sharma et. al., 1993; Battacharya, 1993; Cuq et. al., 2002). These results indicated that the non-leavened control dough had comparable strength with conventional dough (Table 1) in terms of the flow behavior index. A higher flow behavior index for the SCFX-leavened dough showed that supercritical carbon dioxide injection rendered bread dough less vulnerable to increasing shear rates. This is because the gas phase present in the SCFX-dough can deform easily acting as an energy absorber, thus compensating for the shear-thinning effect in comparison with the non-leavened control. The consistency index of the non-leavened control was lower than that for the conventional non-leavened dough (Table 1). This is because the extrusion was performed at the dough proofing temperature, while comparative data on conventional dough was taken at lower temperatures (Table 1). Also, the ingredient formulation that contained bakery fat had a lubricating effect. SCFX- dough had a lower consistency index than the non-leavened control which showed that supercritical carbon dioxide also acted as a diluent, besides being an energy absorber. Differences in the apparent viscosity of the non-leavened control and the SCFX-dough were more pronounced within the low shear rate region. This is because the diluent effect of supercritical

carbon dioxide is more influential than its energy absorbing effect at low shear rates. On the other hand, increase in material throughput caused bubble nuclei to deform more, thus absorbing more energy. Therefore, deformation of bubble nuclei became the governing factor for the rheological behavior at wall shear rates above 38 s^{-1}, suppressing the diluent effect of supercritical carbon dioxide.



Conclusions

Novel SCFX technology rendered continuous production of a half product, the leavened dough. However, baking studies are needed to implement on the overall bread quality upon baking. A derivative benefit of application of SCFX technology to bread dough was the on-line characterization of rheological properties for both the non-leavened control dough and the SCFX-leavened dough with constant history by allowing extrusion at the proofing temperature of classical bread dough and on-line integration of carbon dioxide.

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Effects of heat treatment by using plate heat-exchanger on quality of blood orange juice

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This study was conducted to investigate effects of heat treatment on the quality of Blood orange juice (Citrus Sinensis).

Blood orange juice was pasteurized by continuous flow using plate heat-exchanger (ARMFIELD FT75) at 83.5°c with five holding times: 5.26s, 7.21s, 9.87s, 16.14s and 27.18s.

The most quality parameters were studied: reducing sugar (Lane & Eynon), brix (Atago RX-5000 refractometer), total acidity (Titration with 0.1N NaOH using Titro line 96, schott), vitamin C (Iodine Titration), pH (Inolab 730), color changes: L*, a*, b*, chroma, hue and ΔE (lovibond pfx880), Anthocyanin by PH differential method (results expressed as mg/l of cyanidin-3-glucoside), browning index: absorbance at 420nm (Jasco V- 530 spectrophotometer), clarity transmittance at 650nm, microbial growth (AOAC 2000) and pectin methyl esterase activity (Kimball 1991). Statistical analysis was made for the results using General Linear Model (GLM) test.

The results showed no significant change in pH, total soluble solids and total acidity, the loss were significant in vitamin C content from 45.76 mg/100 ml to 40.1 mg/100ml for the sample holed at 27.18s. The transmittance, a* and chroma decrease significantly, but the browning index, L*(31.01 to 34.1), b* and anthocyanin content increase significantly because of heat treatment. No microbial growth was detected through the lowest holding time, the pectin methyl esterase is inactivated from 88.7% to 95.66%.

Outgassing of flavour compounds during wheat beer fermentation: what is the role of fermentation vessels?

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Bavarian wheat beers have received growing interest during the last decades. According to german law, they have to be brewed with at least 50% wheat malt and the top fermenting "Weissbeer" yeast *Saccharomyces cerevisiae*. Fermentable carbohydrates are converted by yeast into alcohol, carbon dioxide, and numerous other by-products. It is these by-products that have a considerable effect on the taste, aroma, and other properties that characterize the style of beer. In contrast to bottom fermenting yeast strains, *S. cerevisiae* can convert ferulic acid present in malt into 4-vinyl guaiacol, which has a sensory low threshold of 0.3 mg/L and gives to beer a particular phenolic flavor. During fermentation, volatile by-products are partly removed from fermentation vessels by outgasing carbon dioxide leading to finished beers with low concentrations of some flavor active compounds. Therefore, the aim if the present study was to determine the extent of outgasing of some important flavor compounds in dependence on fermentation vessels used in six Bavarian breweries. For this purpose, concentrations of acetaldehyde, ethyl acetate, n-propanol, iso-amyl acetate, iso-butanol and amylalcohols were measured in outgasing carbon dioxide as well as in jung beer at different levels of fermentation. Furthermore, wort samples were analyzed for amino acids, sugars and zinc. The results have revealed that in finished beer a significant relationship exists between concentrations of some compounds and the height of wort in the fermentation vessel used.

Production of isoflavonoids from the soy plant tissue culture using an air-lift bioreactor

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Abstract

Production of isoflavonoids, (Genistein, Daidzein, Genistin, Daidzin) from soy plant tissue culture was investigated in an air-lift biorector. Air-lift biorectors are known for their better biomass and product yields in fermentation of plant tissue cultures due to low shear associated with mixing. PEF (pulsed electric field) was integrated with the air-lift bioreactor. Potential of PEF as an inducer of secondary metabolites, as well as enhancing recovery of invaluable intracellular products was investigated. Influence of air bubble size and rate of aeration based on percentage soluble oxygen was assessed. Effect of PEF (physical stress factor) and addition of methyljasmonate (chemical stress factor) were studied. PEF and methyljasmonate that had a positive effect on Genistin and Daidzin production in small batches did not have similar effects on scale-up in an air lift bioreactor. In batch studies, mixing in a vibrating incubator provided adequate oxygen supply and homogeneity. Increase in culture volume on scale-up necessitated aeration. Growth of the soy plant tissue culture had a thickening effect, thus a higher rate of aeration was required for mixing and oxygen supply. This caused higher shear stress that caused damage to the soy plant cells and prevented them to stay in aggregate form.

Introduction

Plant tissue cultures can synthesize invaluable phytochemicals in conjunction with more than 30 000 chemical bonds (Zhong, 2001) including natural chemicals, secondary metabolites and antibodies. However, production of phytochemicals by plants depends on geographical location, climatic conditions, seasonal variations, growth conditions. In-vitro biosynthesis of secondary metabolites are independent of: geographical location, climatic conditions, seasonal variations, growth conditions. In-vitro biosynthesis of these phytochemicals by plant tissue cultures allows mass production, and thus a continuous supply-demand relationship. Isoflavonoids are secondary plant metabolites that belong to phenylpropanoids (Teuscher, 1990). Isoflavonoids are characterized by branching of a C_6-C_3-C_6 skeleton (Forkmann & Heller, 1999). They are found as either glucose conjugates (Genistin, Daidzin) or in aglucone form (Genistein, Daidzein). They are abundant in soybeans. Soy plant isoflavonoids are known be anti-carcinogenic, and are claimed to offer cancer prevention (Barnes, 1998). Isoflavonoids are claimed to have positive effects on cardiovascular diseases and osteoporosis (Knight & Eden, 1996). Isoflavonoids are known as effective antioxidants (Bourgaut et. al., 2001). Small scale fermentation of the soy plant tissue culture in flasks showed that PEF (physical stress factor) and methyljasmonate (chemical stress factor) induced isoflavonoid biosynthesis (Gueven & Knorr, 2011; Kneer et. al., 1999). It was also found that PEF also enhanced product recovery due to reversible cell membrane permeability (Gueven & Knorr, 2011). On the other hand, plant tissue cultures are known to lose their productivity during scale-up design (Ritterhaus et. al., 1990). However, air-lift bioreactors were reported to provide better biomass and product yields due to low shear associated with mixing (Smart and Fowler, 1984). Air bubbles blown at the bottom plate of the reaction vessel provide mixing and also supply the cells with oxygen (Merchuk, 1990).

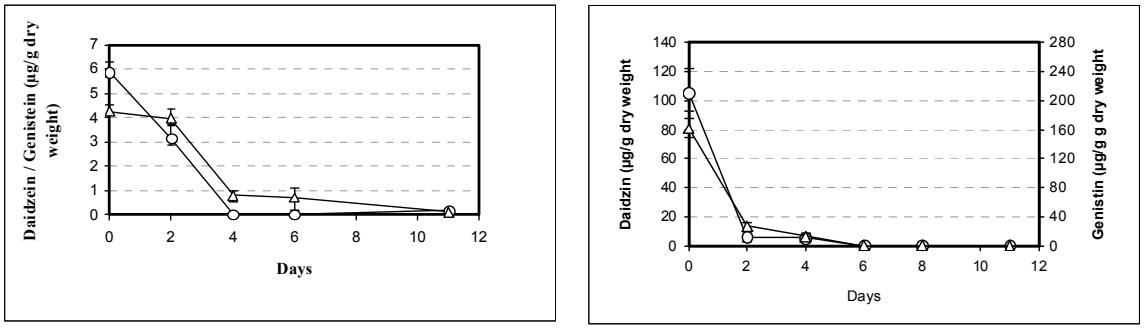
Materials and methods

Glycine max tissue culture suspension was obtained from Federal Research Center for Cultivated Plants - Julius Kühn Institute – Berlin, and was propagated in B_5 medium (Gamborg et. al., 1968). Chemicals

needed for the vitality test (TTC-Test) (Towill and Mazur, 1974) were: 2,3,5-triphenyltetrazoliumchloride (Merck, Darmstadt, Germany), ethanol (Merck, Darmstadt, Germany), and sodium dihydrogenphoshate (Merck, Darmstadt, Germany). HPLC Standards (Roth, Karlsruhe, Germany) were: Daidzein (7,4`- 2 hydoxyisoflavon; Molecular Weight: 254), Genistein (5,7,4`- 3 hydroxyisoflavon; Molecular Weight: 270), Daidzin (7-O-Glycosyl-4`Hydroxyisoflavon Molecular Weight: 416), Genistin (7-O-Glycosyl-4`,5-2 hydroxyisoflavon; Molecular Weight: 432). Methanol (CH4O) (Merck, Darmstadt, Deutschland), acetonitril (C_2 NH_3) (Fisher Chemicals, Loughborough, UK), tetrabutylhydroquinone (TBHQ) (Flucka, Neu-Ulm, Germany) and hydrochloric acid (HCl) (Merck, Darmstadt, Germany) were the other chemicals used in preparation of the standards. Methyljasmonate (95 %) (Sigma-Aldrich Chemicals GmbH, Deisenhofen, Germany) was used as a chemical stress factor to assess its potential as an inducer of isoflavonoids in an air-lift bioreactor. HPLC (Gynkothek, Munich, Germany) equipped with $2x10^{-6}$ m^3 sample bottles (2-7064, Supelco, USA), an RP C-18 separation column (Merck, Darmstadt, Germany), a special software (Chromeleon, Germany) and a UV Detector (Deuteriumlampe L613-08, Hamamatsu, Japan) was used for isoflavonoid analysis. Conductimetry (WTW, Weilheim, Germany) and a spectrophotometry (Uvikon 922, Kontron Instruments, Italy) were used to assess culture vitality. The soy plant tissue culture was propagated in B_5 medium, and then supplied to the air-lift bioreactor under aseptic conditions. Biomass development, electric conductivity and pH of the fermentation medium, culture vitality, membrane permeability of the cells and isoflavonoid concentrations were monitored.

Results and discussion

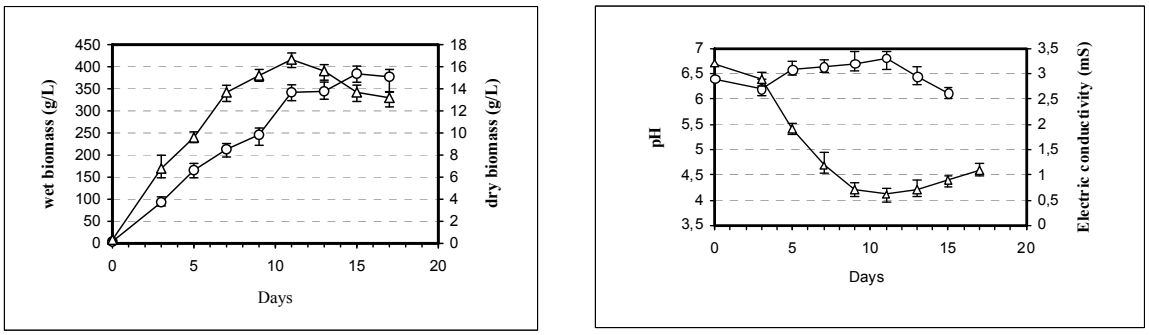
Isoflavonoid concentration decreased during fermentation of the soy plant tissue culture in an air-lift bioreactor (Figure 1). It seemed that movement of large air bubbles through the fermentation medium caused the destruction of the cell aggregates due to excessive shear. The soy plant tissue culture developed as expected upon air bubble size control, and the culture reached maximum biomass (db) and minimum electric conductivity levels on day 11 (Figure 2). Electric conductivity decreased with growth due to the uptake of ammonium and nitrate ions by the cells, essential for their growth. The vitality curves showed that course air bubbles caused complete destruction at Day 9, while the culture survived for 15 days with finer air bubbles (Figure 3). Finer air bubbles also supported better isoflavonoid biosynthesis compared to the culture obtained for course air bubbles (Figures 1 and 4).



(a) **(b)**

Figure 1. Change in isoflavonoid concentration in the air-lift bioreactor **(a)** Daidzein and Genistein **(b)** Daidzin and Genistin

Cells stressed by 1600 volts PEF application on the second day started growing again in 2 days and their mass (db) increased 4 times in the next 48 h (Figure 5). The soy plant tissue culture reached stationary phase 9 days after methyljasmonate addition (Figure 6). Methyljasmonate that increased isoflavonid biosynthesis tremendously during small scale fermentations had no positive effect in the air-lift bioreactor. In the presence of methyljasmonate, PEF application resulted in 50 % decrease in biomass weight (db) (Figures 5 and 7). Constant electric conductivity means no usage of ions, thus inadequate cell development (Figure 7). Inadequate development of the soy plant tissue culture led to inadequate isoflavonoid biosynthesis as expected.



(a) **(b)**

Figure 2. Biomass growth due to aeration by bubble size control **(a)** Growth curve **(b)** pH and electric conductivity

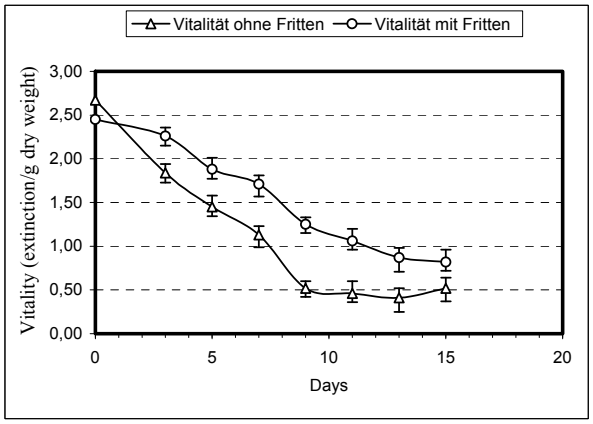
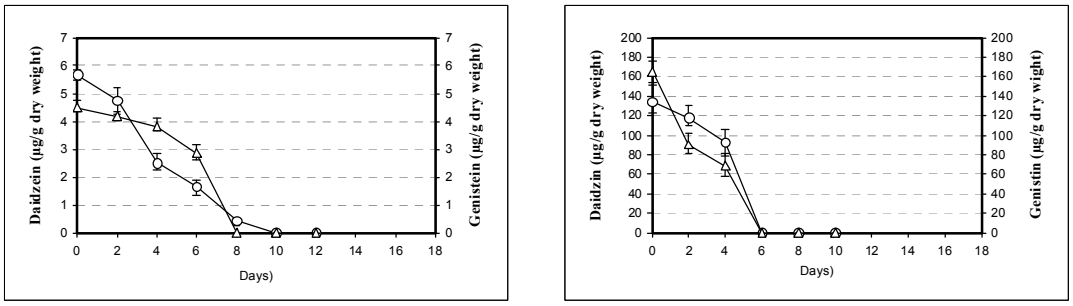
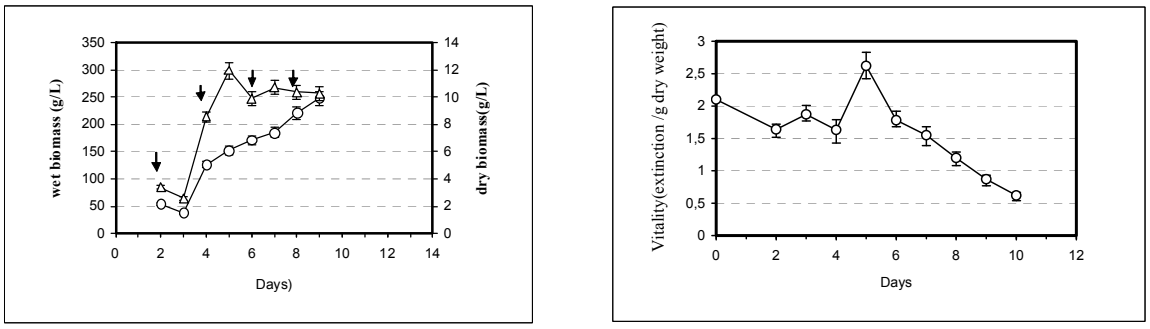


Figure 3. Effect of air bubble size control on the soy plant tissue culture



(a) **(b)**

Figure 4. Change in isoflavonoid concentration after bubble size control **(a)** Daidzein and Genistein **(b)** Daidzin and Genistin



(a) **(b)**

Figure 5**.** Biomass growth after 1600 volts PEF application on Day 2. 4. 6 and 8 as indicated by the arrows **(a)** Growth curve **(b)** vitality

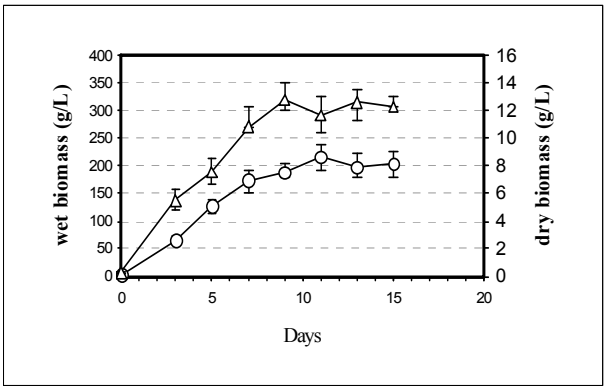
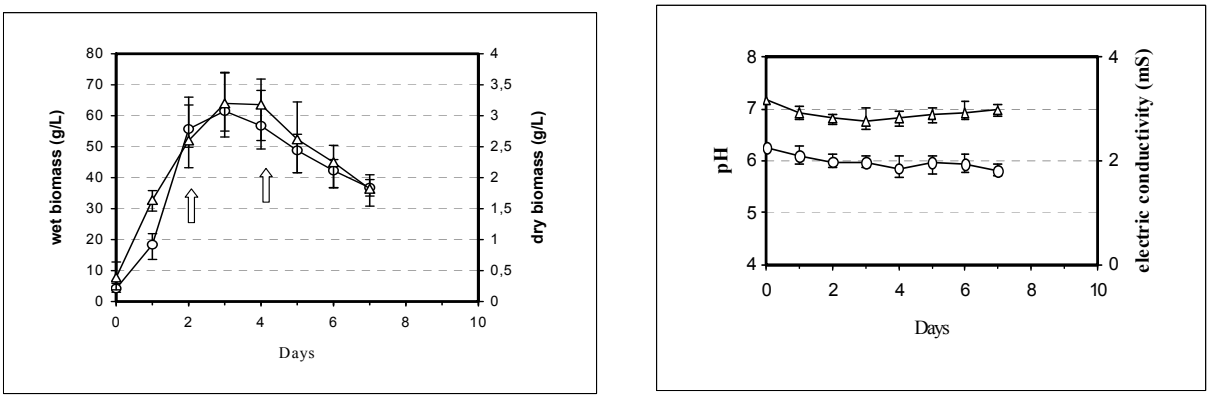


Figure 6 Biomass growth due to methyljasmonate addition



(a) **(b)**

Figure 7. Biomass growth due to combined effect of PEF and methyljasmonate **(a)** Growth curve **(b)** pH & electric conductivity

Cells stressed by 1600 volts PEF application on the second day started growing again in 2 days and their mass (db) increased 4 times in the next 48 h (Figure 5). The soy plant tissue culture reached stationary phase 9 days after methyljasmonate addition (Figure 6). Methyljasmonate that increased isoflavonid biosynthesis tremendously during small scale fermentations had no positive effect in the air-lift bioreactor. In the presence of methyljasmonate, PEF application resulted in 50 % decrease in biomass weight (db) (Figures 5 and 7). Constant electric conductivity means no usage of ions, thus inadequate cell development (Figure 7). Inadequate development of the soy plant tissue culture led to inadequate isoflavonoid biosynthesis as expected.

Conclusions

Shear stress due to aeration prevented the cells from staying in aggregate form. An optimum dilution ratio in a continuous system that does not allow undesired thickening can further be investigated**.**

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Immobilization of hydrolases on semi-permeable membrane surfaces by using polyethyleneimine for industrial applications

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In food industry, many enzymatic and/or non-enzymatic processes require selectively permeable membranes in order to enhance product yield and quality in industrial scale. It is crucial to design a novel membrane with catalytic activity to separate desirable components that can't be effectively separated according to their molecular size. Catalytic membrane reactor systems offer great advantages such as eliminating pressure drops and easy adaptation to any food processes over packed bed reactors in bio-product formation.

In this study, a novel method of β-galactosidase immobilization involving polyethyleneimine (PEI)-enzyme aggregate formation and growth of aggregates on the cellulose acetate (CA) membrane leading to multilayer immobilization of the enzyme was developed. Semi-permeable CA membrane surfaces were created by low pressure plasma polymerization technique. A large amount of β-galactosidase enzyme from *Aspergillus oryzae* was immobilized with 66 % efficiency. The immobilized enzyme showed a good storage and operational stability. The half-life for the immobilized enzyme on the membrane was ~1 month at 30 ºC and ~60 hours at 60 °C, respectively. This immobilized enzyme technology should have important applications in food industry such as oligosaccharide (especially galactooligosaccharides from lactose and fructooligosaccharides from sucrose) production and biodiesel and interesterification processes. The method is simple and straightforward and requires no sophisticated expertise, unlike many other enzyme immobilization techniques.

The combined system based on simultaneous production and separation with high enzyme loading resulted in high reactor productivity. This system can be easily adapted to the other food processes which require effective separation of inhibitory substances reducing the product yield and purity.

Sourdough fermentation and evaluation of gliadin degradation

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In genetically susceptible individuals, with the consumption of wheat, rye and barley, an autoimmune disease occurred in small intestine that is called celiac disease. A life-long gluten-free diet is the only treatment and it leads to the recovery of small intestine mucosa. Therefore, variety of gluten-free products is of great importance for these patients in order to follow this strict diet. Since lactic acid bacteria (LAB) have proteolytic capacity, sourdough, fermented dough contains LAB naturally, draws attention. During sourdough fermentation, gluten proteins can be degraded by both LAB and endogenous proteolytic enzymes of wheat flour. On the other hand improved texture, flavor, shelf-life and nutritional value can be the positive outcomes of sourdough fermentation.

The objective of this study was to perform sourdough fermentation with LAB, and to evaluate fermentation parameters and degradation of gliadins during sourdough fermentation. Individual and mixed cultures of *Lactobacillus acidophilus, Lb. casei* and *Lb. delbrueckii* ssp. *bulgaricus* were used in wheat flour sourdough formulations and fermentation parameters were followed during 48 h. Dough sample which was acidified with the mixture of lactic and acetic acid was also prepared in order to investigate the effect of acidity. SDS-PAGE and 2-D electrophoresis were applied to gliadins extracted from dough samples.

After fermentation, pH decreased to 3.84-3.52, total titratable acidity reached the 13.49-17.34 range, and the LAB population was counted as 10^7-10^9 cfu/g dough. Results of SDS-PAGE and 2-D electrophoresis showed that degradation observed in gliadin fractions during fermentation period. Obtained gliadin band and spot modifications revealed that degradation was due to the wheat flour endogenous enzymes which became active under acidic condition that was provided by LAB.

Studies on process factors affecting sodium value of apple juice concentrate

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High levels of dietary sodium are associated with raised blood pressure and adverse cardiovascular health. Fruit juices also include some amount of sodium. In this study, effects of process factors are studied in industrial production of clear juice concentrate. The sodium level of apple juice increased during extraction and clarification steps. In order process water, bentonite, CIP chemicals mostly affect the sodium level of apple juice concentrate.

Gelatinization Properties of Soft and Hard Syrian Wheat

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Abstract

Two Syrian Hard wheat samples and two Syrian Soft wheat samples were used in this study. The samples were brought from The General Company of Mills in Homs. The research included a comparison of the gelatinization properties of the studied flour and starch samples using Brabender Micro Visco Amylo-Graph, and a study of the correlation between gelatinization properties and protein and damage starch content. Syrian Soft wheat flour had significantly ($P \leq 0.05$) higher Breakdown and time at the beginning of gelatinization than those of Syrian Hard wheat flour. Syrian Hard wheat flour had significantly ($P \leq 0.05$) higher time and temperature at the Maximum viscosity than those of Syrian Soft wheat flour.

Starch source (Hard, Soft wheat) wasn't significantly correlated with starch gelatinization properties. Soft wheat starch had significantly higher Maximum viscosity and, time and temperature at the beginning of gelatinization than those of Soft wheat flour. Whereas, Soft wheat flour had higher Breakdown than Soft wheat starch. Hard wheat starch had significantly higher time and temperature at the beginning of gelatinization than those of Hard wheat flour. The flour content of both protein and damage starch were negatively correlated ($P \leq 0.05$) with the torque and temperature at the Beginning of gelatinization, and Breakdown, in contrast, there were positive correlations at 5% level of significance between the content of both protein and damage starch, and time and temperature at the Maximum viscosity.

Key words: Gelatinization, hard wheat flour, soft weat flour, viscosity

Introduction

Wheat flour is the main ingredient in most breads, bakery products, biscuits, cookies, crackers, noodles and processed foods such as prepared breakfast cereals, sauces, gravies, soups, confectionery products and flour tortillas.[1]

The most important trait that determines how a particular wheat cultivar is processed is grain hardness (texture). Grain hardness forms the fundamental basis of commercial differentiation. Texture determines flour particle size, starch damage, water absorption and milling yield. Therefore, grain hardness is an indicator of the suitability of particular flour for a particular product. The hardness of the grain is determined by the adhesion of the endosperm's protein matrix to the starch granules. Due to strong adhesion, starch granules fragmentize during milling of hard wheat. This fragmentation is called starch damage. Starch damage is the most important factor in determining water absorption of flour. It also determines the amount of carbohydrates available to yeasts for fermentative activity. Therefore it affects positively gas production, loaf volume and, as a result, baking quality.[2]

Starch is the primary component of wheat (*Triticum aestivum* L.) flour, and consequently, plays an important role as a determinant of food product quality. Starch exists naturally in the form of discrete granules within plant cells. The starch granule is mainly composed of amylose and amylopectin [4,3]. Gelatinisation is a process that occurs when starch granules are heated in the presence of water, gelatinisation is not a single reaction but a process with a number of irreversible reactions including swelling of the starch granules, leakage of amylose from the starch granules, loss of crystallinity, and during this process, the secondary bonds that maintain the granule structure are broken and the micellar network is pulled apart. Gelatinisation takes place over a temperature interval and is influenced by factors such as the starch source, water content, presence of damaged starch, starch isolation procedure, annealing and environmental conditions during growth [5, 6, 7].

As heating continues and more and more granules become swollen, the viscosity of the medium increases, a maximum viscosity is reached when the largest percentage of swollen intact granules is present which is referred to as peak viscosity, at this point, the starch is considered to be fully pasted. For native starches, continued heating eventually results in a decrease in viscosity as the granules dissolve and the polymers are solubilised. Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of the granular compounds and total disruption of the granules [8,9].

Starch pasting properties are the main wheat compositional factors determining wheat processing quality and the textural attributes of diverse wheat-based products as breads, noodles, and cookies [10], starch gelatinization is the key reaction in the cooking of wheat grains, with steam under pressure which is the initial process for some breakfast cereals [11]. Starch gelatinization during pasta drying has a major contribution to pasta quality [12]. The thrust of starch technology in the 'diet' and 'health' sector of the food industry has been to support the development of new wholesome and balanced foods without sacrificing the desirable characteristics associated with the traditional high-calorie fat-containing food products. In fat-reduction programmes, the key functions of starches are in achieving a significant calorie reduction coupled to the rebuilding of features which hitherto would have been provided by the fats in the product. These are viscosity, body and mouth coating [6].

Several methods can be used to follow the gelatinization process: loss of birefringence, increase in viscosity, increase in enzyme susceptibility, decrease in enthalpy, and loss of X-ray diffraction pattern. Of these, only the measurements of enzyme susceptibility and variation of enthalpy are used to quantify the extent of gelatinization. These methods however, are either time-consuming (enzyme susceptibility) or do not show good reproducibility.[5] Pasting properties of starches are measured by using various instruments such as Brabender Visco-Amylograph, Rapid Visco Analyser, Ottawa starch viscometer, and Haake viscometer. Among these instruments, BVA and RVA are the most commonly used instruments [13].

Even with the better analytical power of BVA, the large quantity of sample and long testing time limits its application, to overcome this disadvantage, a new model of Micro Visco-Amylo- Graph was developed. This instrument is designed on similar principles as the BVA, but differs in sample size, cooking speed, and spindle design [13].

Materials and Methods

The Syrian Hard and Soft wheat samples were brought from The General Company of Mills in Homs. Wheat samples were tempered and milled using Buhler MLU-202 mill by AACC, 2001 methods, starch was isolated by a dough kneading procedure [19]. The starch was air-dried. Flour moisture was determined by Method 44-15A (AACC 1999). Damage starch percent was determined using Chopin-Mixolab by [15,16]. Starch nitrogen was determined by ICC, Standard 2001, No: 105/1. Falling number by ICC, Standard 1995, No: 107/1. Wet and dried gluten by ICC, Standard 1994, No:155/1.

Flour and starch suspensions of 13% (dsb) were used for the MVA test., 15.0 g (dsb) of flour and starch was directly weighed and100 ml water was added. Suspensions were cooked at 7.5°C/min cooking rate and stirred at 250 rpm rotation speed. Suspensions were heated from 30°C to 92°C at 7.5°C/min, held at 92°C for 1 min, cooled to 55°C at 7.5°C/min, and held at 55°C for 1 min. Data were analyzed using Minitab program14.0.

Results and discussion

Gelatinization properties are summarized in Table 1, some of the flour gelatinization properties were significantly correlated to wheat type (Hard,Soft). Syrian Soft wheat flour had significantly ($P \leq 0.05$) higher Breakdown and time at the beginning of gelatinization than those of Syrian Hard wheat flour. Syrian Hard wheat flour had significantly ($P \leq 0.05$) higher time and temperature at the Maximum viscosity than those of Syrian Soft wheat flour.

No significant correlation existed between other flour gelatinization properties and wheat type. Breakdown for Soft wheat flour was significantly larger than that for Hard wheat flour. It is therefore evident that the starch granules in Soft wheat flour would become less resistant to thermal treatment.

Table 1. Gelatinization properties of flour samples*

Flour / Property	Soft I	Soft II	Hard I	Hard II
	Mean ± St.D			
Temperature at the beginning of gelatinization(C ̊)	60.7^{c}±0.15	60.8^{bc}±1.10	59.2^{ab}±0.65	58.1^{a}±0.95
(BU) Beginning of gelatinization	12.7^{a}±0.58	13.0^{a}±1.00	11.7^{a}±1.16	11.7^{a}±0.58
(min) Time at the beginning of gelatinization	4.2^{c}±0.03	4.2^{c}±0.03	4.1^{b}±0.03	3.9^{a}±0.07
Maximum viscosity(BU)	411.0^{b}±8.19	387.7^{a}±2.08	441.0^{c}±7.00	413.0^{b}±1.73
viscosity(min) Time at the Maximum	8.0^{b}±0.03	7.9^{a}±0.03	8.2^{c}±0.00	8.3^{d}±0.03
Temperature at the Maximum viscosity (C°)	89.3^{b}±0.21	88.3^{a}±0.40	91.1^{c}±0.10	91.6^{c}±0.72
setback(BU)	185.3^{c}±3.51	172.7^{abc}±17.62	167.0^{b}±6.56	146.0^{a}±8.54
Breakdown(BU)	115.3^{b}±16.04	109.3^{b}±2.31	64.7^{a}±3.79	53.7^{a}±6.66

*All samples were analyzed in triplicate and St.D was computed.
*Same letters mean no significant difference at P≤0.05.

Correlation coefficients between the flour gelatinization properties and content of both protein and damage starch are shown in Table. 2.The flour content of both protein and damage starch were negatively correlated (P≤0.05) with the torque and temperature at the Beginning of gelatinization, and Breakdown, in contrast, there were positive correlations at 5% level of significance between the content of both protein and damage starch, and time and temperature at the Maximum viscosity.

Table 2. Correlation coefficients between the flour gelatinization properties and content of both protein and damage starch*

Property	TBG	BG	TIBG	MV	TIMV	TMV	Setback	Breakdown
Protein %	-0.98	-0.97	-	-	1.00	0.99	-	-0.95
Damage starch UCD	-0.99	-0.97	-	-	0.99	0.99	-	-0.96

* TBG: Temperature at the beginning of gelatinization, BG: Beginning of gelatinization, TIBG: Time at the beginning of gelatinization, MV: Maximum viscosity, TIMV: Time at the Maximum viscosity, TMV: Temperature at the Maximum viscosity. * significant at P≤0.05.

The results shown in Table.3 indicate that, starch source (Hard, Soft wheat) wasn't significantly correlated with starch gelatinization properties.

Table 3. Gelatinization properties of starch samples*

Flour / Property	Soft I	Soft II	Hard I	Hard II
	Mean ± StD			
Temperature at the beginning of gelatinization (C ̊)	66.0 ^{b}± 0.12	65.8^{b}±0.29	67.1^{c}±0.58	64.7^{a}±0.29
(BU) Beginning of gelatinization	12.0^{b}±0.00	11.0^{a}±0.00	10.7^{a}±0.58	10.7^{a}±0.58
(min) Time at the beginning of gelatinization	4.7^{b}±0.04	4.9^{c}±0.06	4.8^{bc}±0.08	4.6^{a}±0.06
Maximum viscosity(BU)	451.0^{b}±10.4	460.7^{b}±2.31	466.0^{b}±3.46	400.0^{a}±10.39
viscosity(min) Time at the Maximum	8.2^{ab}±0.13	8.3^{a}±0.04	8.3^{b}±0.00	8.5^{c}±0.04
Temperature at the Maximum viscosity (C°)	90.9^{ab}±0.98	91.2^{a}±0.174	92.1^{b}±0.12	92.5^{c}±0.17
setback(BU)	113.7^{a}±21.94	174.7^{c}±8.08	159.7^{b}±2.31	145.3^{bc}±23.67
Breakdown(BU)	44.7^{c}±0.58	37.7^{b}±7.51	80.0^{c}±12.12	32.3^{ab}±1.16

*All samples were analyzed in triplicate and St.D was computed.
*Same letters mean no significant difference at P≤0.05.

The results in Table(4) shown that, soft wheat starch had significantly higher Maximum viscosity and, time and temperature at the beginning of gelatinization than those of Soft wheat flour. Whereas, Soft wheat flour had higher Breakdown than Soft wheat starch.

Table 4. Gelatinization properties of soft wheat starch and flour samples*

Sample / Property	Soft Wheat I		Soft Wheat II	
	Starch	Flour	Starch	Flour
TBG(Cº)	66.0 ^{b} ± 0.1	60.6 ^{a} ± 0.2	65.8 ^{b} ± 0.3	60.7 ^{a} ± 1.1
BG(BU)	12.0 ^{a} ± 0.0	12.6 ^{a} ± 0.6	11.0 ^{a} ± 0.0	13.0 ^{b} ± 1.0
MV(BU)	451.0 ^{b}± 10.3	411.0 ^{a} ± 8.2	460.6 ^{b} ± 2.3	387.6 ^{a} ± 2.08
TMV(Cº)	90.8 ^{a} ± 0.9	89.3 ^{a} ± 0.2	91.2 ^{b} ± 0.2	88.2 ^{a} ± 0.4
Setback(BU)	133.6 ^{a} ±21.9	185.3 ^{b} ±3.5	174.6 ^{a} ± 8.1	172.6 ^{a} ±17.6
Breakdown(BU)	44.6 ^{a} ± 0.6	115.3 ^{b} ±16.0	37.6 ^{a} ± 7.5	109.3 ^{b} ±2.3
TIBG(min)	4.8 ^{b} ± 0.04	4.2 ^{a} ±0.05	4.8 ^{b} ± 0.06	4.2 ^{a} ± 0.04
TIMV(min)	8.2 ^{a} ± 0.1	7.9 ^{a} ± 0.06	8.2 ^{b} ± 0.04	7.8 ^{a} ± 0.08

* Same letters mean no significant difference at P≤0.05.

* TBG: Temperature at the beginning of gelatinization, BG: Beginning of gelatinization, TIBG: Time at the beginning of gelatinization, MV: Maximum viscosity, TIMV: Time at the Maximum viscosity, TMV: Temperature at the Maximum viscosity

The results shown in Table.5 indicate that hard wheat starch had significantly higher time and temperature at the beginning of gelatinization than those of Hard wheat flour.

Table 5. Gelatinization properties of hard wheat starch and flour samples *

Sample / Property	Hard wheat I		Hard wheat II	
	Starch	Flour	Starch	Flour
TBG(Cº)	67.0 ^{a} ± 0.6	59.1 ^{a} ± 0.6	64.6 ^{b} ± 0.3	58.1 ^{a} ± 0.9
BG(BU)	10.6 ^{a} ± 0.6	11.6 ^{a} ± 1.2	10.6 ^{a} ± 0.6	11.6 ^{a} ± 0.6
MV(BU)	466.0 ^{b} ± 3.5	441.0 ^{a} ± 7.0	400.0 ^{a} ± 10.4	413.0 ^{a} ± 1.7
TMV(Cº)	92.0 ^{b} ± 0.1	91.1 ^{a} ± 0.1	92.5 ^{a} ± 0.2	91.5 ^{a} ± 0.7
Setback(BU)	159.6 ^{a} ± 2.3	167.0 ^{a} ± 6.5	145.3 ^{a} ± 23.6	146.0 ^{a} ± 8.5
Breakdown(BU)	80.0 ^{a} ± 12.1	64.6 ^{a} ± 3.8	32.3 ^{a} ± 1.2	53.6 ^{b} ± 6.6
TIBG(min)	4.82 ^{b} ± 0.08	4.1 ^{a} ± 0.02	4.6 ^{b} ± 0.06	3.9 ^{a} ± 0.06
TIMV(min)	8.3 ^{b} ± 0.0	8.2 ^{a} ± 0.0	8.4 ^{b} ± 0.04	8.2 ^{a} ± 0.03

* Same letters mean no significant difference at P≤0.05.

* TBG:Temperature at the beginning of gelatinization, BG: Beginning of gelatinization, TIBG: Time at the beginning of gelatinization, MV: Maximum viscosity, TIMV: Time at the Maximum viscosity, TMV: Temperature at the Maximum viscosity .

Conclusions

Gelatinisation properties of flour depend on the type of wheat (Soft, Hard), but the gelatinisation properties of the starch are independent on the type of wheat which extracted from, so the wheat flour composition as protein content and damage starch affect on gelatinisation properties of flour.

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Study of PPO activity using microplate reader

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The polyphenol oxidases (PPO) are found in almost all living organisms including plants, animals, bacteria and fungi. Plant polyphenol oxidases are responsible for the enzymatic browning reactions occurring during the handling, storage and processing of fruits and vegetables.

There are three types of polyphenol oxidases which were classified according to their ability to oxidase different types of phenolic compounds. Tyrosinase (monophenol monooxygenase E.C. 1.14.18.1) oxidizes monophenols such as tyrosine, p-cresol and p-coumaric acid but not diphenols and triphenols. Second type of polyphenol oxidase (1,2- benzenediol: oxygen oxidoreductase; E.C 1.10.3.1) is also known as polyphenolase, phenolase, catechol oxidase, cresolase, or catecholase mostly found in higher plants especially mushroom, apple, peach, tobacco and tea leaves. These enzymes in higher plants and fungi oxidize a great variety of monophenolic and o-diphenolic compounds and catalyze two types of reactions. First reaction involves the hydroxylation of a monophenol to give a diphenol and second reaction involves the removal of hydrogens from diphenol to give quinone. There is another enzyme, laccase (E.C. 1.10.3.2) most often found in fungi, catalyzes the oxidation of monophenols, o- and p-diphenols, aminophenols and diaminoaromatic compounds.

In enzymology three techniques were generally used to determine the activity of PPO: HPLC, polarography and spectrophotometry. But there are different difficulties during using these techniques to determine the PPO activity such as the high volume, the rapidity of the substrate oxidation, and the difficulty in the control of temperature and homogeneity of the reaction medium which make the precedent techniques inaccuracy in determination of PPO activity.

This study describes a rapid simple accurate automated method to measure the activity of laccase activity produced from *Myceliophtora thermophila*. The apparatus measures the absorbance of colored end-products due to the oxidation of phenolic substrats (ferulic acid and catechol), the changes in absorbance by the time describe the enzymatic activity. This apparatus is equipped of 96-well plat, so it can carry out of high number of enzymatic reactions (96 experiments) in micro volumes that can reach to 300µl in the same time. Furthermore, the apparatus can control the shaking intensity and the temperature of the reaction medium.

The results of this study showed the importance of use of microplate reader to measure the activity of laccase because it can decrease the time of measurement (up to 15 times), the experiment coasts and the possibility to make high number of experiments in the same controlled conditions with minimum errors.

The importance of this research is due to exploit the microplat reader in a novel application that differs from the others as immunological and enzymological tests, and it confirms the importance of replacement of traditional spectrophotometric apparatus by the microplate reader apparatus to determine the enzymatic kinetic that depends on colored reactions.

Thermal stability enhancement of raspberry (*Rubus idaeus L.*) *and* dewberry (*Rubus caesius L.*) anthocyanins by copigmentation with mandarin and pomegrate peels

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Abstract

In this research raspberry and dewberry fruits were used. Oven dried pomegranate (rich in tannins) and mandarin (rich in hesperidin, rutin) peels were used for copigmentation. Peel powder was added to the juices diluted with acidified water (ABS 1.00) at 2% (w/v) ratio. HPLC-DAD chromatograms were obtained for anthocyanins and phenolics. After overnight copigmentation absorbances were spectrophometrically measured. Bathochromic shift ($\Delta\lambda_{max}$=10 nm) and a hyperchromic effect (ΔABS_{max}=5-13) were observed indicating copigmentation. Thermal degradation kinetics were examined at 70, 80 and 90 ºC which was calculated as first order. The half-life of raspberry anthocyanins were calculated as 23, 12 and 5 hours which increased to 29, 14 and 10 hours upon pomegranate peel addition with respect to 70, 80 and 90ºC. Dewberry anthocyanins have almost identical half-lives as that of raspberry. Pomegranate copigmentation increased half-life periods to 29, 17 and 8 hours. Mandarin peel showed significant effect only at 90ºC for both fruit samples. Thermal activation energies (E_a) were calculated as 18.85 kcal/mol for raspberry which decreased to 13.61 and 15.85 kcal/mol upon copigmentation with pomegranate and mandarin peels, respectively. The E_a=17.14 of dewberry anthocyanins decreased to 12.02 and 16.32 kcal/mol after copigmentation with pomegranate and mandarin peels, respectively.

Key words: Raspberry, dewberry, anthocyanins, thermal stability, copigmentation

Introduction

Color of the food is one of the most important factors affecting consumer preference. Food industry uses colorants to enhance the appearance of foods. Anthocyanins, well-known natural alternatives to synthetic dyes, are the pigments of many fruits and vegetables responsible for red, purple and blue colors [1].

Factors that affect the stability of anthocyanins include structure, pH, temperature, light, copigments, self-association, metallic ions, oxygen, ascorbic acid, sugars and their degradation products, proteins and sulfur dioxide. Researches conducted recently, indicate that the main mechanism providing color stability in plants is the molecular interaction between anthocyanins and copigments. The most effective way of copigmentation is the acylation of aromatic and/or aliphatic acids onto the anthocyanidin nucleus through a sugar molecule (inte-molecular copigmentation). Another copigmentation form, on the other side, is the inter-molecular non-covalent interaction between anthocyanin and copigment molecules (hydrophobic complex formation). As a result of copigmentation the color density of anthocyanin extracts (hyposochromic effect) and their maximum wavelength (bathochromic shift) increase [2, 3].

The anthocyanins present in raspberries are derivatives of cyanidins; cyanidin-3- rutinoside, cyanidin-3 sophoroside, cyanidin-3-glucosylrutinoside and cyanidin-3-glucoside. Since there are no acylated anthocyanins present in raspberries and due to high –OH presence in cyanidins, they show low thermal stability [4]. The anthocyanins present in dewberries are are mainly cyanidin-3-glucoside, cyanidin-3-rutinoside and malvidin-3-glucoside which also have low thermal stability [5].

In this research raspberry and dewberry extracts copigmented with dried pomegranate amd mandarin peel powders were studied for their thermal stability.

Materials and methods

Preparation of anthocyanin extracts

Raspberry (*Rubus idaeus* L.) and dewberry (*Rubus caesius* L.) were obtained from local markets in Bishkek. Samples were blended (Waring laboratory blender, USA) and then centrifuged at 4000 rpm for 15 minutes (Labofuge, Heraus 400, Germany). The juice was filtered (Whatman no. 4) and necessary dilutions were carried out with acidified water containing 0.01% H_2SO_4.

Copigmentation of anthocyanin extract

Pomegranate and mandarin peels were cut into small pieces and oven dried at 60°C over night (Memmert, Germany). The peels were then powdered in a porcelaine mortar. Peel powders were added in a 2% (w/v) ratio into anthocyanin exracts. After 24 hours of copigmantation the absorbances and the maximum wavelength of the samples were measured (Pelkin-Elmer Lambda EZ-201).

HPLC analysis

Agilent 1200 series HPLC system was used equipped with UV-DAD detector set to 280 and 520 nm. A C-18 column (250 mmx4.6 mm i.d. ACE 5 μm) was used. Solvent A: 100% acetonitrile; solvent B: 4% phosphoric acid in water was used with a linear program of 6%A at 0. min; increasing to 25%A at 18.33 min. The flow rate of the mobile phase was 1.00 mL/min.

Thermal stability measurement

Copigmented samples and references were placed in a temperature-controlled water bath (Memmert, Germany) at 70, 80 and 90°C. Five mL of samples were poured in screwed glass vials and placed in water bath. Vials were removed in 15 minutes intervals for the first hour, then in 30 minutes intervals for 640 minutes. First order degradatiom kinetics were applied.

Results and Discussion

The maximum wavelengths of the anthocyanin extracts of raspberry and dewberry increased from 510 nm to 520 nm upon copigmentation. Type of copigment, pomagranate or mandarin peel did not affect the amount of bathochromic shift. However, higher hyposochromic effect was observed with dewberry samples regardless of the copigment type. (Table 1).

Table 1. Maximum wavelength and absorbances before and after copigmentation in raspberry and dewberry extracts

Sample	λ_{max}=510 nm	λ_{max}=520 nm
	ABS (t=0 h)	ABS (t=24 h)
Mandarin peel+dewberry	0,499	0,512
Mandarin peel+raspberry	0,538	0,545
Pomegranate peel+ dewberry	0,504	0,515
Pomegranate peel+raspberry	0,547	0,552

HPLC chromatograms for copigmented rapberry and dewberry anthocyanin extracts did show new peak formation indicating non-colavent weak interactions (data not shown).

Thermal degradation rates of both reference and copigmented samples were evaluated using first order kinetics. Reaction rate constants (k), half-life periods ($t_{1/2}$) and activation energies (E_a) are given in Table 2.

Table 2. Degradation reaction rate constants (k), half-life periods and activation energies (E_a) for;

a. Dewberry extracts

Sample	T (ºC)	*k* (1/min)	$t_{1/2}$ (h)	E_a (kcal/molK)
Reference	70	0.0005	23	17.14
	80	0.0010	11,5	
	90	0.0020	6	
Pomegranate peel copigmentation	70	0.0004	29	12.08
	80	0.0007	17	
	90	0.0015	8	
Manderin peel copigmentation	70	0.0006	19	16.32
	80	0.0008	14	
	90	0.0016	7	

b. Raspberry extracts

Sample	T (ºC)	*k* (1/min)	$t_{1/2}$ (h)	E_a (kcal/molK)
Reference	70	0.0005	23.0	18.85
	80	0.0010	11.5	
	90	0.0023	5.0	
Pomegranate peel copigmentation	70	0.0004	28.8	13.62
	80	0.0008	14.4	
	90	0.0012	9.6	
Mandarine peel copigmentation	70	0.0005	23.0	15.85
	80	0.0010	11.5	
	90	0.0018	6.4	

Copigmentation affected the thermal stability of both, raspberry and dewberry, extracts. For both samples, addition of mandarin peel powder did not increase the thermal stability of the anthocyanins significantly. On the other hand, pomegranate peel as copigment showed significant affect on thermal stability of the anthocyanin extracts. Thermal activation energies (E_a) were calculated as 18.85 kcal/mol for raspberry which decreased to 13.61 and 15.85 kcal/mol upon copigmentation with pomegranate and mandarin peels, respectively. The E_a=17.14 of dewberry anthocyanins decreased to 12.02 and 16.32 kcal/mol after copigmentation with pomegranate and mandarin peels, respectively.

Preliminary experiments with other fruit peels did not give promising results due to interaction with the red color of anthocyanins. Mandarin and pomegranate peels were chosen since no interaction was observed. HPLC chromatograms showed that both bear high level of phenolics (chromatograms at 280 nm) which did not overlap with anthocyanin peaks of both raspberry and drewberry extracts. This study showed that copigmentation occurs between the phenolics present in the peels and the anthocyanins of the extracts. Also, there is an increase in thermal stubility due to copigmentation. Further studies are planned to investigate the phenolics of pomegranate and mandarin peels involved in copigmantation of raspberry and drewberry extracts.

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Shelf-life of apples coated with whey protein concentrate-gellan gum edible coatings

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Abstract

Edible coatings made from whey protein concentrate (WPC) and gellan gum (G) were investigated for their capacity to preserve the quality of *Malus domestica* cv Golab apples. WPC and gellan gum coatings at different concentrations, plasticized with glycerol (Gly) were tested. Postharvest storage quality conditions tested included weight loss, color and texture changes, titratable acidity and soluble solids content and consumer acceptance.

Result indicated that WPC-gellan-coated fruits were rated highest for taste, glossiness, colour and overall acceptability and lowest for weight loss in this study.

Results also showed no significant difference in soluble solids content and titratable acidity between control and coated apples after 4 weeks at 4°C.

Key words. Apple, edible coating, whey protein concentrate, gellan gum, shelf-life

Introduction

Edible coatings, which are defined as thin layers of wax or other material applied to the surface of a food, have been used for over 800 years. Records dated as early as the 12th and 13th centuries showed that wax coatings were applied to citrus fruits in China (Hardenburg, 1967). Coatings of edible materials applied as a thin layer to enhance the quality, extend the shelf-life of fruit and work as a barrier reducing both respiration and water loss (Olivas, Mattinson & Barbosa-C´anovas, 2007). Edible coatings can provide an additional protective coating for fresh products and can also give the same effect as modified atmosphere storage in modifying internal gas composition (Baldwin, 1994). Recently, several edible coatings for preserving fruits such as oranges, apples, and grapefruits were successfully applied (Park, 1998). The mechanism by which coatings preserve fruits and vegetables is by producing a modified atmosphere surrounding the product. This modified atmosphere can serve several purposes, including reducing oxygen availability and increasing the fruit or vegetable's internal carbon dioxide concentration (Smith, Geeson, & Stow, 1987). Modified atmospheres created by coatings are produced by the physical trapping of carbon dioxide gas within the fruit tissues during respiration (Ball, 1997).

Edible films have been proven to be an e1ective preservation technique that can not only keep fruit plumpness, fresh appearance and hardness but also improve the luster of fruits' surface thereby increasing the commercial value of fruits (Xu, Xu & Chen, 2003).

More recently waxes and edible coatings made from proteins, polysaccharides, and various combinations of theses products, have been used on many other fruit and vegetable commodities as well as for other food applications including nuts, and meat products (Kester & Fennema, 1986). Such coatings have been used to reduce moisture loss and surface wounding, as well as to reduce a variety of diseases in apple varieties (Hardenburg, 1967; Kester & Fennema, 1986; Bai, Alleyne, Hagenmaier, Mattheis & Baldwin, 2003).

Protein films and coatings possess excellent oxygen barrier properties, comparable to synthetic polymer polyvinylidene chloride and ethylene vinyl alcohol films (Trezza & Krochta 2002).Whey Protein

Concentrate (WPC) edible coatings in combination with anti-browning agents effectively extended the shelf-life of minimally processed apple slices by 2 week when stored in packed trays at cold storage (Lee, Park, Lee, & Choi, 2003).Whey protein-based coatings without incorporation of antioxidants were more effective in reducing enzymatic browning of 'Golden Delicious' apples than hydroxypropyl methylcellulose-based coatings (P´erez-Gago, Serra, Alonso, Mateos, & del R´ıo, 2005).

Many gums and their derivatives have been used for coating purposes. Coatings which have been used for apple coating based on hydrocolloids include: Carboxymethyl cellulose (CMC) dust and starch for freshly cut pieces of fruits (Mason, 1969) and chitosan and lauric acid (pennisi, 1992) for apple slices.

The objectives of this investigation were **1)** to develop methodologies for forming simple protein and composite protein-gum films based on WPC on apple, **2)** to study the shelf life and sensory analysis of coated apples.

Materials and Methods

Materials

WPC (85 percent protein) was supplied by Arla Foods (Videbeak, Denmark).Gellan gum was purchased from Fisher Scientific, Inc. (Fair Lawn, USA). Glycerol and calcium chloride (CC) by Merck (Darmstadt, Germany) was added as a plasticizer to all film-forming solutions.

Fruit selection and preparation

Apples were bought at a local market in Tehran and immediately transported to the laboratory for the experiments. The *Malus pumila* cv Golab was chosen for this experiment because of its short shelf life. Selected apples of uniform size and color were washed in distilled water, dried, and defective ones were eliminated before treatments. Coated and uncoated fruits were kept at 4±1 °C for 28 days.

Experimental Design

Initial baseline values of each tested variable were established on day 0 of the test period using 10 apples. The five coated groups and the control group were subsequently tested every seven days. Weight was measured on the 7th, 14th, 21st and 27th days.

Coating formulations

Five coating treatments were applied to *Malus pumila* cv Golab Apples. The control treatment was no coating. The remaining 5 treatments were variations such as: (T1) 4gr Gellan +1 gr Calcium Chloride + 195 gr DW, (T2) 10gr WPC+0.05 gr Gellan + 90 gr distilled water (DW), (T3) 12gr WPC+3 gr Gly+ 0.05 gr Gellan + 88 gr DW, (T4) 11gr WPC +3 gr Gly+ 89 gr DW and (T5) 4 gr Gellan + 3 gr Gly + 193 gr DW. Coating treatments were made by heat denaturing a 10percent (wt/wt) aqueous solution of WPC for 30 min in a 90 °C water bath (McHugh & Krochta, 1994) (Fig.2). The solutions were cooled to room temperature in an ice bath. The appropriate amounts of glycerol plasticizer were added and stirred for 30 min to achieve total dissolution. Deionized water was used for all solutions.

Soluble solids content and Titratable acidity

Soluble solids content were quantified every 7 days for 28 days in triplicate. Soluble solids in the juice were determined with a refractometer Kruss D-22976 (Hamburg, Germany) using a sucrose scale calibrated at 20°C. The amount of juice obtained decreased during storage as water loss in the apple slices increased. These higher concentrated juices were conducive to higher values of citric acid and soluble solids. Therefore, the amount of soluble solids obtained was compensated for weight loss as follows:

$V = X* (100 - \%WL_t) 100$

Where X is the value for soluble solids obtained from apple juice before weight loss compensation, % WL the percentage of weight loss at time t, and V is the corresponding true value for soluble solids or citric acid content after weight loss compensation.

Titrable acidity analyzed according the method described by Lees (1971) was used for the determination of titratable acidity and the results were expressed as percentage citric acid.

Weight loss determination

Ten fruits for each specific condition were randomly selected and the fruits were weighed during the study with a laboratory weight balance Mettler AE 200-S (Greifensee, Switzerland) per replication. Results were expressed as percentage weight loss.

Sensory evaluation

Fruit colour was measured by the CIE L*a*b* system using a chroma meter Minolta Model CR-300 (Minolta. Co. Ltd., Japan) at 6 h intervals for 24 h.). A white tile (L*: 97.46; a*: -0.02; b*: 1.72) was used as reference. Firmness was evaluated by a puncture test on the sides of the cubes prepared from quarters using a TA-XT2 texture analyser from Stable Micro Systems with The cross head speed was 100 mm/min and the load cell used was 50 kg. The maximum amount of force (load kg) needed to puncture the apple sample was recorded. Two samples per apple were tested and analyzed as subsamples equipped with a rounded 2 mm diameter flat-head steel probe. Peel firmness measurements were taken as the first peak force value obtained during the test to penetrate the fruit 7 mm at 1.5 mm/s and pulp firmness as the medium force. Three samples per apple were tested and analyzed as subsamples.

Statistical analyses

Data were analyzed using factorial analysis in order to determine whether the coated apples' post harvest quality parameters differed from the uncoated control apples. Factorial analysis was also used to determine if any of the parameters tested changed over time. Analysis of variance (ANOVA) calculations were used for the factorial analysis. Days or treatments that differed significantly at the P=0.05 level were subjected to Duncan's difference test to compare each treatment to the nontreated control.

Results and Discussions

Soluble solids content and titratable acidity

Soluble solids content of coated and un-coated apples stored under cold condition decreased at the end of the storage period. The loss of soluble solids during the storage period is natural, as sugars, which are the primary constituent of the soluble solids content of a produce, are consumed by respiration and used for the metabolic activities of the fruits (Özden & Bayindirli, 2002).

The major sugar in 'Golab' apples was fructose which was 1.68±/0.42 gkg^{-1} (fresh weight basis). Coated fruit had lower sugar levels no significant differences were found due to treatments (Table 1). Generally, all apples exhibited an increase, when their initial titratable acidity contents (expressed as citric acid) were compared to final ones at the end of storage period, to varying extents, depending on the applied specific treatment.

There were no significant differences in titratable acidity and soluble solids among the coatings for all of the varieties (data not shown), possibly because of the relatively brief storage time. This is somewhat unexpected. Apples with inhibited respiration generally maintain organic acid levels better during storage than fruit with uninhibited respiration rates (Baritelle, Hyde, Fellman, & Varith, 2001).

Weight loss of coated apple

Edible coatings produced on T3 and T4 were suited to extending lower fruit weight loss (1.3percent). The fruits coated with T1, T2 and T5 solutions presented results similar to the uncoated apples. Weight loss was highest for fruit without any coatings after 14 days and for T2 since second week of storage time. (Figure 1).

Post harvest weight changes in fruits and vegetables are usually due to loss of water through transpiration. This loss of water can lead to wilting and shriveling which both reduce a commodity's marketability. Edible films and coatings can also offer a possibility to extend the shelf life of fresh-cut produce by providing a semi-permeable barrier to gases and water vapor, and therefore, reducing respiration, enzymatic browning, and water loss (Guilbert, 1986; Baldwin, Nisperos-Carriedo, & Baker, 1995).

No shrinkage was detected for WPC-gellan and gellan (T3 and T4) coated fruits (Figure 2). Hatfield and Knee (1988) and Maguire, Banks, Alexander and Gardon (2000) reported that even as little as 3.5-5 percent weight loss can lead to shrivel in apples. Only T3 and T4 apples lost lower than 1.5percent of initial weight, which is considered not enough to induce shriveling.

Sensory evaluation

Table 3 shows the sensory evaluations of apples in different coatings and uncoated apples. The result of sensory evaluation conducted on uncoated (control) and coated apples, after 28 days of storage. In colour measurement, the score for T3 and T4 was significantly higher than for the fresh sample, while those for other treatments were significantly similar uncoated apples (Fig. 3). Although some T2 samples showed significant brownish dots or streaks on the flesh; this is difficult to detect with the colorimeter, which integrates the entire surface exposed for measurement, probably diluting the visible incipient browning effect.

After 28 days the control and sample T1, T2 and T5 had a loose skin and peeled easily with knife and even with hand from fruit flesh. Samples T3 and T4 had a firm skin. Texture loss is the most noticeable change occurring in fruits and vegetables during prolonged storage and it is related to metabolic changes and water content (Garcı´a, Martino´, & Zaritzky, 1998).

Taste of T3 and T4 samples was significantly ($P<0.05$) higher than other treatments and uncoated apples. In T3, apples gave significant difference in the firmness, crunchiness and overall acceptability compare with other treatments and control.

Table 1. Average soluble solids and acid citric content of coated apples and un-coated (control) after 28 days cold storage.

Sample	**S.S (%)**	**citric acid (g/kg)**
Control	11	1.68
T1	9	1.4
T2	11	1.4
T3	10	1.4
T4	9	1.3
T5	11	1.3

Table 2. Sensory attributes of apples coated with WPC/Gellan gum edible coatings and uncoated (control) apples after 4 week at 4°C.

Sensory attribute	Control	T1	T2	T3	T4	T5
Taste	6.16 ± 0.06^{a}	4.33 ± 0.23^{b}	4.27 ± 0.37^{b}	7.50 ± 0.56^{c}	7.00 ± 0.06^{c}	4.14 ± 0.02^{b}
Glossiness	6.33 ± 0.40^{a}	4.88 ± 0.12^{b}	4.48 ± 0.62^{b}	7.14 ± 0.42^{c}	6.58±0.26c	5.12 ± 0.44^{b}
Sweetness	6.06 ± 0.40^{a}	5.88 ± 0.12^{a}	6.18 ± 0.62^{a}	7.14 ± 0.42^{b}	5.58±0.26a	6.12 ± 0.44^{a}
Overall acceptability	5.04 ± 0.08^{b}	5.32 ± 0.14^{b}	$5.450.12^{b}$	8.02 ± 0.54^{c}	6.86 ± 0.30^{d}	5.23 ± 0.52^{b}

Means with same superscript are not significantly different ($P > 0.05$)

Table 3.Color attribute for basic material (BM) at day 0 vs uncoated and coated apples at 28th day

	L*	b*	FMAX
BM	83.6±0.44	42.3±0.34	3.6±0.30
Uncoated	67.2±0.30	44.3±0.47	1.1±0.20
T1	68.7±0.45	47.12±0.50	2.3±0.10
T2	65.6±0.18	45.1±0.57	1.8±0.10
T3	79.8±0.80	58.2±0.40	2.8±0.08
T4	76.26±0.22	56.21±0.37	2.1±0.06
T5	65.82±0.50	48.6±0.24	1.6±0.11

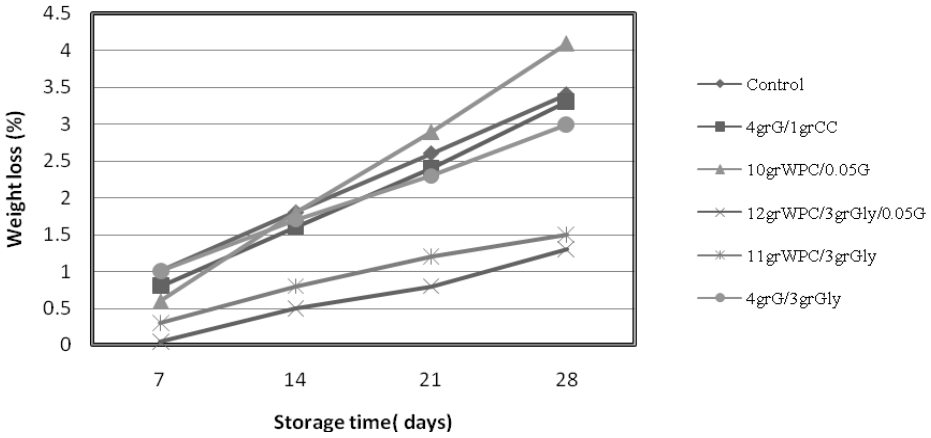
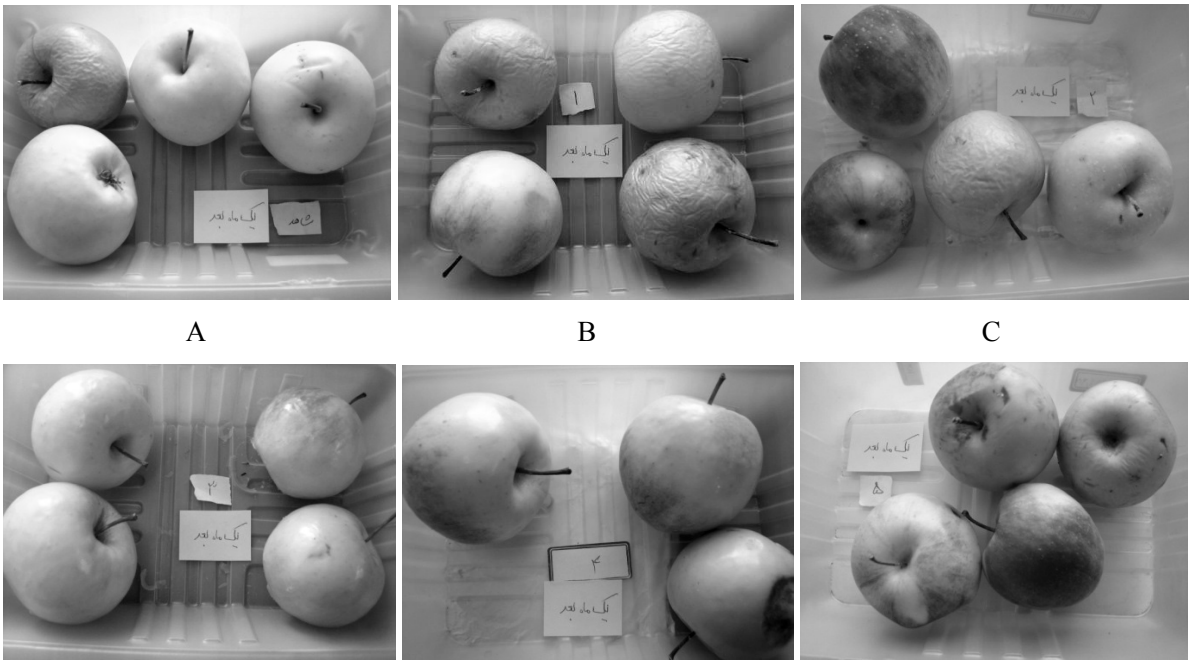


Figure 1. Weight loss evolution in apple samples during cold storage period. Data shown are the means (6standard deviation).



A B C

D E F

Figure 2. Changes in gloss, shrinkage and shriveling in coated (A) and un-coated (B: T1, C: T2, D: T3, E: T4 & F: T5) apples after 28 days storage in 4±1 °C and 70± 2 % relative humidity.

Conclusion

The present work studied the effects of dipping in innovative coating solutions on apple in cold storage. WPC-gellan coatings with added plasticizer effectively maintained color, Firmness, glossiness and Overall acceptability of apple during storage. The collected data showed that in uncoated apple samples sensory attributes were lower than in coated samples. No differences were detected in the changes in chemical parameters (weight loss, soluble solids and citric acid) values between both samples during storage.

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Integrated lethality for the non-ideal flow of a pseudoplastic liquid food

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Abstract

The analysis of the continuous thermal processing of a liquid food can be compromised by the common assumptions of isothermal ideal flow, instant heating and cooling steps and minimum residence time approach. In order to optimize safety, sensorial and nutritional attributes of the food, the thermal process should be modeled considering flow, heat transfer and kinetics principles. In this work, the thermal processing of a pseudoplastic (power-law) fluid in a tubular system is modeled taking into account the effective heat diffusion and mass diffusion in laminar flow, the reaction kinetics for microorganism or enzyme inactivation and quality attribute changes, as well as heat losses to the ambient. The model comprises differential equations for heat and mass balances applied to the heating and cooling sections (double-pipe heat exchangers) and the holding tube. Axial and radial components were discretized using finite difference methods and the model was solved using gPROMS (PSE). Main simulation results were the radial and axial distributions of temperature and concentration of microorganisms. The axial distribution of the integrated lethality (S-value) was also obtained for process evaluation. The model was successfully applied for the study of the pasteurization of soursop juice and the results are presented and discussed.

Introduction

The continuous thermal processing of liquid foods was developed to solve problems observed in the batch processing, such as low rate of heat penetration and long processing time to achieve the correct temperature needed to ensure the required lethality. The main advantages of continuous thermal processing, when compared to discontinuous processing, are: increasing production capacity, reduction of energy consumption and improvement in the sensorial characteristics of the final product due to lower processing temperature and time (Torres and Oliveira, 1998).

Pseudoplastic liquid foods like fruit juices and purées have a complex flow behavior and are often processed in heat exchangers in laminar flow. This way, the velocity profile, the residence time distribution and the temperature profile, regarding axial and radial domains, need to be considered for proper lethality evaluation and design of the thermal process (Gratão, 2006).

Despite these flow characteristics, the assumptions that are often used for the process design are: isothermal plug flow at the maximum velocity (minimum residence time), instant heating and cooling (the lethality takes place only in the holding tube) and no mass or heat dispersions or heat losses to the ambient. Since these assumptions lead to over-processing, they can guarantee product safety but undesired nutritional and sensorial changes of the product may occur (Jung and Fryer, 1999).

Nowadays, the demand for processed foods goes beyond basic requirements of food safety and assurance of shelf-life stability. Recent studies have been conducted to determine the most appropriate conditions of thermal processing in order to preserve sensorial and nutritional characteristics of the food product (Ditchfield *et al.*, 2006; Awuah *et al.*, 2007).

The objective of this work was to develop and test a mathematical model to simulate the continuous thermal processing of a pseudoplastic liquid food under non-ideal laminar flow in order to determine the integrated lethality and the temperature profile.

Materials and Methods

The mathematical model was developed considering a double-pipe heat exchanger composed by heating section, holding tube and cooling section. Differential mass and energy conservation equations were used in the mathematical modeling with appropriate boundary conditions for each control volume (Fogler, 2006). The variables were discretized using finite difference methods and the model was solved using gPROMS (Process System Enterprise, version 3.2) considering 400 axial points per section and 30 radial points for the variable discretization.

The non-Newtonian fluid (power-law) contains a general "component A" which follows first order thermal destruction kinetics. This general component could represent a microorganism, an enzyme activity or a nutrient concentration. The assumptions for this fluid modeling were as follows: incompressibility, developed laminar steady-state flow, radial diffusion of mass (component A), effective radial disffusin of heat, uniform physical properties in each section and negligible viscous dissipation.

The heating or cooling fluid flows in the heat exchnager annulus, counter-currently regarding the food product. For the simulations, hot and cold water were considered as fluid utilities for the heating and cooling sections, respectively. The assumptions for both were: incompressibility, developed turbulent flow (plug-flow), uniform physical properties in each section, thermal axial dispersion and negligible viscous dissipation.

The equipment exchanges heat with the ambient due to natural convection of the air. A layer of thermal insulation was considered and modeled for the holding tube, but the outer surface of the heating and cooling sections were in direct cointact with the ambient air.

The axial dimensionless domain, η, varied from 0 to 1 in the heating section, from 1 to 2 in the holding tube and from 2 to 3 in the cooling section. The radial dimensionless domain, present in the food product control volume, varied from 0 (tube center) to 1 (tube wall).

Equation 1 was incorporated in the model to represent the velocity profile of a non-Newtonian fluid (power-law model) flowing under laminar regime in a circular section tube (Toledo, 1999):

$$v_{product} = \bar{v}_{product}\left(\frac{3n+1}{n+1}\right)\left(1 - x^{\frac{n+1}{n}}\right) \quad (1)$$

where: n = flow behavior index (dimensionless), $v_{product}$ = product velocity ($m \cdot s^{-1}$), $\bar{v}_{product}$ = average product velocity ($m \cdot s^{-1}$) and x = radial domain (dimensionless).

Equation 2 was used for the evaluation of the effect of the thermal processing on the product according to the number of decimal reductions of component A, S_{value} (Toledo, 1999).

$$S_{value} = \log\left(\frac{C_{Ao}}{C_A}\right) \quad (2)$$

where: C_A = flow-average concentration of component A (mixing cup), C_{A0} = inlet concentration of A and S_{value} = number of decimal reductions (dimensionless).

Regarding the dispersion of heat and of component A through the product, it was considered that the radial mass Peclet number was equal to the radial thermal Peclet number.

Results and Discussion

To test the simulation model, the thermal processing of 18 ºBrix soursop juice (pseudoplastic fluid) with a flow rate of 18 L h^{-1} was studied. The thermal inactivation of yeast and molds ($D_{82.2ºC}$ = 0.57 s, z = temperature gradient to reduce D by a factor of 10 = 7 ºC) was used to evaluate the process lethality. It was

considered a small-scale heat exchanger with the following dimensions: internal diameter of 4.5 mm and each section with 5 m length (heating, holding tube and cooling).

The inlet temperature of the hot water was set to obtain a flow-average product temperature of 74 ºC at the end of the holding tube (process parameter). The thermo-physical properties (density, specific heat and thermal conductivity) and rheological properties (consistency index and flow behavior index) of the food product were defined using appropriate equations considering the estimated average temperature in the specific section of the heat exchanger. It was admitted that the Peclet number (radial and axial) for the soursop juice was 2000 and the Peclet number (axial) for the heating and cooling water was 1300 in all simulation cases.

Figure 1 shows the obtained temperature profile of the soursop juice in the heating section (tube center and tube wall). Due to the laminar regime and rheological properties, the tube center takes longer to have its temperature increased than the tube wall. For instance, halfway through the heating section ($\eta = 0.5$), the temperature at the center and at the tube wall is 59 ºC and 74 ºC, respectively.

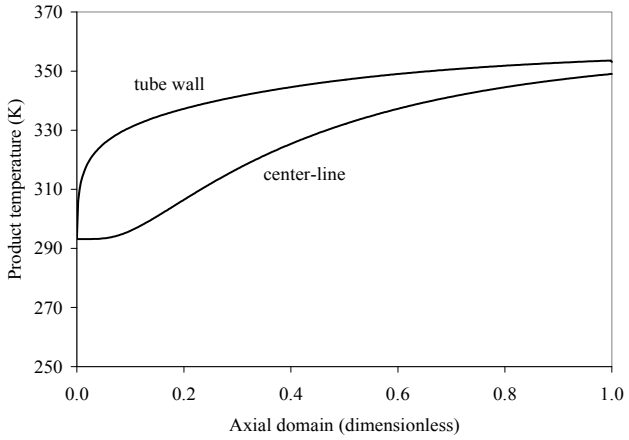

Figure 1. Axial profile of the food product temperature in the heating section

Since the thermal destruction rate is strongly temperature dependent, the concentration of yeasts and molds is higher at the center of the tube than at the wall as shown in Figure 2. Halfway through the heating section (2.5 m) the concentration of micro-organisms at the center of the tube decreased to approximately half of the initial value. Regarding the tube wall, the same concentration was only achieved at 3.3 m of the heating section.

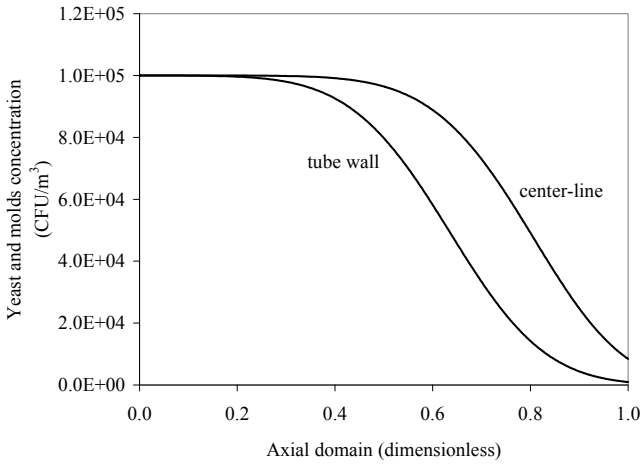

Figure 2. Axial profile of the concentration of yeast and molds in the heating section

Figure 3 illustrates the effect of considering the dispersions (mass and thermal) and the heat losses to the ambient in the simulation results regarding the center-line temperature. When the dispersions are neglected, the radial heat transfer rate is low and it takes longer to heat or cool the product than when the dispersions are taken into account. When heat losses are neglected, the required inlet temperature of the hot water is lower; therefore, the product wall temperature is also lower.

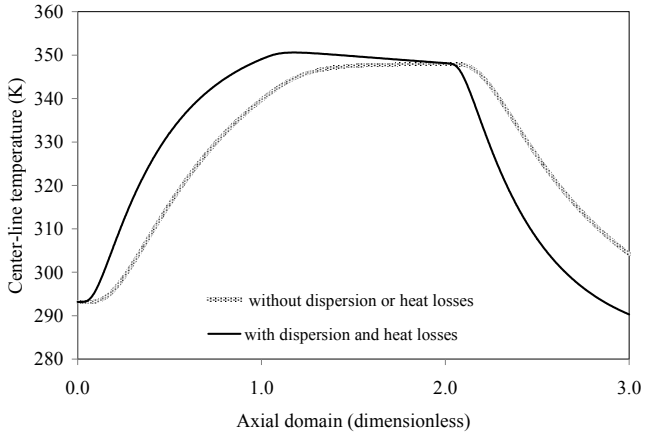

Figure 3. Effect of the model assumptions on the center-line temperature

Figure 4 shows the effect of the model assumptions on S_{value}. When it was considered that the process was isothermal with plug flow at maximum velocity with the lethality only in the holding tube, the S_{value} was the smallest. On the other hand, taking into account the dispersions, heat losses and velocity profile in all sections of the heat exchanger, the S_{value} was strongly affected. A holding tube with approximately 19 m (instead of 5 m) would be necessary to obtain an S_{value} of 5.74 if it was considered that the process was isothermal at the maximum velocity with only the holding tube. The longer time that the soursop juice should need to be submitted to high temperature would impact negatively in its sensorial and nutritional characteristics.

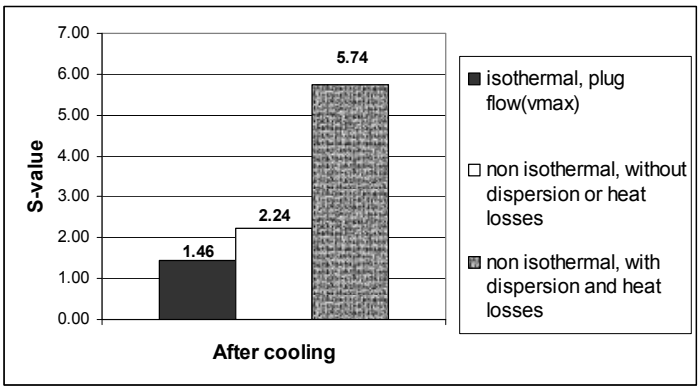

Figure 4. Effect of model assumptions on the number of decimal reductions S_{value}

The separate effects of the thermal and mass dispersions assumptions on the distributed lethality can be analyzed in Figure 5. When just thermal dispersion was considered, the yeast and molds did not disperse radially and the effect of the temperature on the average concentration of the micro-organisms was lower (S_{value} at the end of cooling section = 4.36) than when all the assumptions were taken into account (S_{value} at the end of cooling = 5.74). Since the heat transfer rate is lower when only mass dispersion and the thermal condutivity (instead of effective thermal conductivity) are considered, it was necessary to increase the inlet hot water temperature. Consequently, the S_{value} was highest for this case (S_{value} at the end of cooling = 6.02).

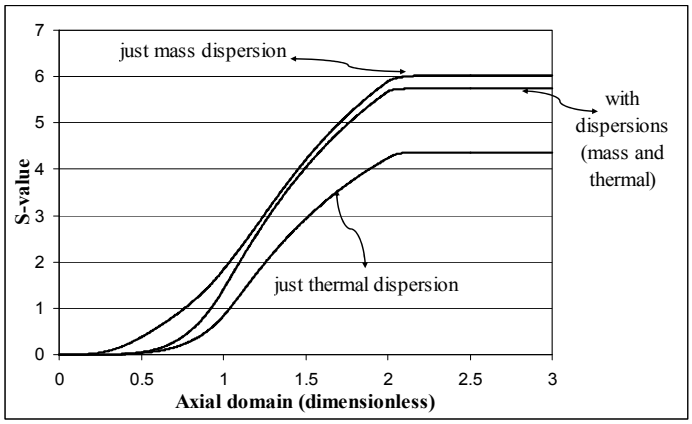

Figure 5. The effect of considering mass or thermal dispersions on S_{value} (based on the average concentration)

Figure 6 presents the effect of the product flow rate on S_{value}. It can be seen that, the higher the flow rate of the soursop juice, the lower is the S_{value}, even considering that the inlet temperature of hot water was 1 ºC higher in the case of the higher flow rate. This can be justified by the fact that the faster the flow the shorter the time that the fluid is exposed to high temperature.

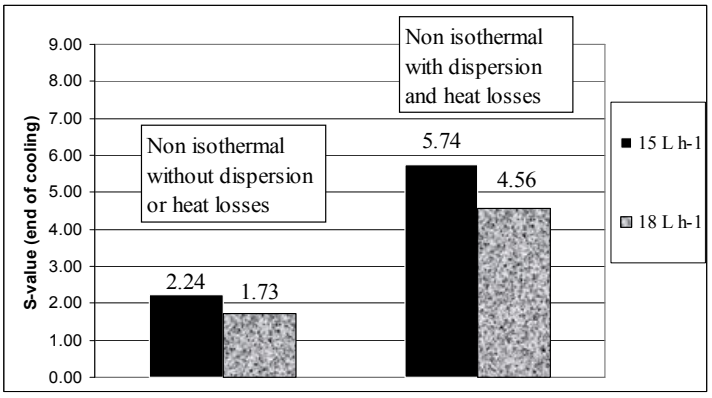

Figure 6. The impact of the food product flow rate on S_{value} (end of cooling section)

The effect of the soursop soluble solids content on S_{value} was evaluated and the results are shown in Figure 7. When the food product has 12 ºBrix, the required inlet temperature of hot water was 1 ºC lower than when the concentration was 18 ºBrix, probably due to the higher heat transfer rate for the less concentrated fluid. For the same concentration, the S_{value} was approximately 2.5 times higher when the dispersion (mass and thermal) and heat losses are considered in the modeling than when they are neglected.

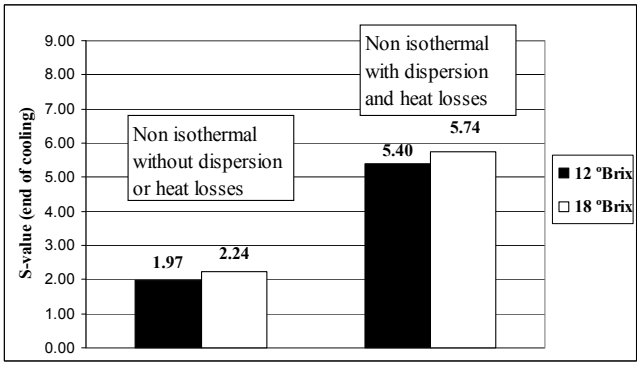

Figure 7. The effect of the product solible solids concentration on S_{value} (end of cooling)

Conclusions

A mathematical model was developed to simulate the thermal processing of a viscous non-Newtonian fluid in a tubular heat exchanger. Thermal and mass dispersions, as well as heat loses do the ambient, were considered in the model. The model was tested for the study case of soursop juice and it was possible to determine the temperature and the lethality distributions throughout the equipment. The effect of the model assumptions on the simulation results was analyzed and a large difference was obtained in comparison with the conventional model of isothermal flow at maximum velocity. The effect of the product flow rate and soluble solids concentration were also studied. As consumer demands for products with better sensorial and nutritional characteristics is increasing, proper models are required for process design and evaluation in order to guarantee the product safety with minimal processing and maximum nutrient and sensorial retention.

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Mathematical modeling approaches for thermal processing operations

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Modeling studies in thermal processing operations are of greater importance since thermal processing is the most common technique used for preservation of foods, and it is the critical control point to determine the process efficiency and to ensure safety.

Modeling a thermal processing operation is an interdisciplinary approach that might involve engineering approaches for transport phenomena driving the process with chemistry, reaction kinetics and predictive microbiology. Essential aspects of a thermal processing model are to define physical, chemical or biological changes during the process, to develop mathematical basis of the process with appropriate assumptions, to solve the problem with required mathematical background and to validate the model for various processing conditions. Therefore, a mathematical model to simulate the thermal processing operation must be physics based describing the transport phenomena (formulation of continuity, energy and momentum equations with initial and boundary conditions) occurring during the process.

In this review, physics-based mathematical model approaches for modeling of thermal processing operations will be explained focusing on heat transfer and fluid dynamics which might be encountered widely in different food processing operations. For this purpose, analytical solutions, numerical approaches and CFD methodologies bringing new innovative approaches into the modeling will be described briefly with different thermal processing examples and model validation techniques.

Mathematical modeling of pistachio drying (*cv. Ohadi*)

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The pistachio nut (*Pistachia vera* L.) is one of the favorite nuts of the world that is widely cultivated in saline, dry and hot areas of the Middle East, Mediterranean countries and USA. Due to high nutritional value and favorable taste, pistachio nut has great economic value for Iran, and for countries that import this product. proper harvest and good processing are the major basis of having good quality. Drying of agricultural products has always been of great importance for the preservation of food by human beings. cabinet drying are well-known food preservation techniques that reduce the moisture contents of agricultural product, and thereby prevents deterioration within a period of time regarded as the safe storage period. Since all fresh pistachios can not be consumed at the time of harvest, preservation provides a larger market, allowing consumers to buy the preserved pistachio around the year. Simulation models are helpful in designing new or in improving existing drying systems or for the control of the drying operation. In this reaserch quality properies of pistachio in during of drying were measured in different tempreture(50, 60,70 and 80°C) by a laboratory cabinet drier.

Shrinkage and rehydration ability of food materials during air drying adversely affects the quality of the dried products. The experimental shrinkage data show a linear behavior with moisture content, with a reduction in sample volume with decreasing moisture content. The effect of temperature and air velocity on the shrinkage phenomenon, the colour and the rehydration ability can be neglected. The experimental data didn't show the dominating effect of temperature on them. Behavior effectively. The change of material moisture content in the falling rate drying period is for investigation of drying characteristics of pistachio nuts, it is important to model the drying proportional to the immediate difference between material moisture content and the expected material moisture content that it comes into equilibrium with the drying air. The numerical model was developed to simulate the moisture transfer during of drying of a spherical pistachio nuts by finite difference method on MATLAB software. The predicted water loss content was validated by comparison with the experimental values ($R2 > 0.99$). It can be concluded that this model describes well the mechanism of moisture diffusion in a one-directional transfer during drying of a spherical pistachio nuts.

Effect of spray drying parameters on properties of spray dried yoghurt powder

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Abstract

Yoghurt is the most popular fermented milk in the world. Yoghurt, being a highly perishable product, has a limited shelf-life; the primary objective of drying is to preserve the yoghurt in a high quality shelf-stable powder form without need for subsequent refrigeration. The nutritional value of yoghurt powder is due to it containing less lactose and it has the potential to enhance the immune response, lower plasma cholesterol levels, and enhance mineral solubility and bioavailability. Generally, yoghurt is dried by freeze drying or spray drying. Spray-drying is a well-known process suitable for yoghurt drying because it allows preparation of stable and functional products. In the present study samples of yoghurt with 11.5% constant total solids concentration were used as feed. A pilot scale spray dryer with mixed air/feed flow system and a two-fluid nozzle atomizer was employed for the spray drying process. In all experiments, drying air flow rate, the feed rate, the feed temperature, compressed air flow rate were kept at 500 m^3/h, 2±0.1 l/h and 30 ±0.5˚C, 1 bar respectively. The variable parameter were inlet air temperatures 150,170,190±0.1˚C and the yoghurt powders were analyzed for moisture content, bulk density, particle density, dispersibility and color parameters. Analysis of experimental data yielded correlation between the powder properties and the variable operating conditions. It was observed that powder moisture content, density and L decreased and dispersibility increased with an increase in air inlet temperature.

Key word: yoghurt, spray drying, powder properties

Introduction

Yoghurt is the most popular fermented milk in the world. This is partly attributed to many desirable effects of the product that are readily accepted by consumers and also due to the image of the product as 'healthy' (Wirjantoro and Phianmongkhol, 2009). Yoghurt is a highly nutritious protein-rich product obtained by fermentation of milk with *S. thermophilus* and *L. bulgaricus.* The nutritional and therapeutic effects of yoghurt are well known and mainly attributed to fermentative changes in the milk and/or the metabolic effects of the yoghurt microflora (Venir *et al*.,2007).

Spray drying is the transformation of feed from a fluid state into a dried particulate form by spraying the feed into a hot drying medium (Goula & Adamopoulos, 2005). Spray drying has a high moisture removal rate, lower cost, and shorter process time. It also allows preparation of stable and functional products and can be implemented for large-scale throughputs (Koc et al., 2010).

The primary objective of drying is to preserve the yoghurt in a high quality shelf-stable powder form without need for subsequent refrigeration. Yoghurt powder of high quality without the need forrefrigeration. Dried yoghurt requires less packaging and storage costs because of the reduction of bulk, and no refrigeration is required (Kumar and Mishra, 2004). It can be used as an ingredient for baked goods, confectioneries, poultry feed, instant yoghurt, and starter culture.viable cell number is an important factors of yoghurt quality (Tamime and Robinson, 1999).

The most commonly quoted specifications of a powder involve moisture content, bulk density and solubility. The temperatures and drying conditions experienced by a droplet during drying have an important influence on the above powder properties (Goula & Adamopoulos, 2005).

The aim of this work was to study the influence of different inlet air temperatures on physicochemical properties of the powder produced, including moisture content, bulk density, particle density, solubility index, dispersibility and color.

Materials and Methods

Spray drying

A pilot scale spray dryer with mixed air/feed flow system and a two-fluid nozzle atomizer was employed for the spray drying process. In all experiments, drying air flow rate, the feed rate, the feed temperature, compressed air flow rate were kept at 500 m^3/h, 2±0.1 l/h and 30 ±0.5˚C, 1 bar respectively. 3 inlet air temperatures were investigated, i.e.150, 170, 190˚C. The design of the dryer is such that the outlet air temperature, controlled to the inlet temperature, cannot be set with a temperature regulator (Table 1). All the spray dried powders were collected in container then sealed in dark bottle and stored at 4 ˚C.

Table 1. Effect of different inlet temperature (◦C) on outlet temperature (°C)

Inlet temperature (◦C)	Outlet temperature (◦C)
150	63.30±0.94
170	69.8±1.55
190	80.90±0.93

Analysis of powders

Moisture content

The measurement of the yoghurt powder moisture content was carried out by drying 1±0.1 g of yoghurt powder in a hot-air oven (Galenkamp, England) at 100±1°C for 2 h. The moisture content was then calculated, using the following equation (AOAC. 2000).

$$\text{Moisture content (\%)} = \frac{\text{loss of sample weight during dry}}{\text{initial sample weight}} \times 100$$

Bulk density

Yoghurt powder was transferred to a 50 ml graduated cylinder. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder (Goula & Adamopoulos, 2005).

Particle density

The powder sample (25 g) was transferred into a 100 ml measuring cylinder with a glass stopper. Then 50 ml of petroleum ether was added and the measuring cylinder was shaken until all the powder particles were suspended. Finally, all the powder particles on the wall of the cylinder were rinsed down with a further 10 ml of petroleum ether (60 ml in total) and the total volume of petroleum ether with suspended powder was read (Jinapong *et al.*, 2008). The particle density was calculated as follows:

$$\rho_{particle} = \frac{\text{Weight of powder (g)}}{\text{total volume (ml) - 60}}$$

Dispersibility

13 g of yoghurt powder was stirred with 100 g of water at 40 ±2°C in a beaker. Stirring was carried out to make 25 complete back and froth movements across the whole diameter of the beaker for 15 s. The reconstituted yoghurt powder was then poured through the sieve of 210 μm size. The sieved yoghurt (10 ml) was transferred to a weighed and dried dish and dried for 4 h at 105°C (A/S Niro Atomizer, 1978c).The dispersibility of yoghurt powder was measured using following equation:

$$\text{dispersibility\%} = \frac{(100 + a) \times TS\%}{a \times \frac{100 - b}{100}}$$

Where, a = amount of powder (g) being used, b = moisture content in the powder, and % TS = dry matter in percentage in the reconstituted soymilk after it has been passed through the sieve.

Color

L*, a* and b* parameters were determined using a digital imaging (sony, model DSC-S500, Japan) and photoshop software that L* is the lightness or darkness (L = 0, black; L = 100, white), $_{+}$a is redness, $_{-}$a is greenness, +b* is yellowness, and -b* is blueness.

Results and Discussion

Moisture content

The moisture content of the yoghurt powders varied from 2.8% to 4.59% water base. The results showed that at constant feed flow rate and air flow rate the moisture content of the spray-dried powders decreased with increasing in inlet and outlet air temperature (Table 2). The results were calculated from three replicate measurements.

This is because of higher inlet temperature; the rate of heat transfer to the particle is greater, providing greater driving force for moisture evaporation. Consequently, powders with reduced moisture content are formed (Quek *et al*., 2007). The results were consistent with other funding (Goula *et al*., 2004). Generally, in a spray drying system, the temperature of the exhaust air leaving the drying chamber controls the residual moisture in the powder. A lower moisture content can be reached by higher temperatures at the outlet (Goula & Adamopoulos, 2005). Each data in the Table2 represents the averaged values of three determinations.

Density

Bulk density varying from 0.42 to 0.39 g/cm^3. Table 2 shows powder bulk density in relation to inlet air temperature. Increased air inlet temperature causes a reduction in bulk density, as evaporation rates are faster and products dry to a more porous or fragmented structure. According to Walton (2000), increasing the drying air temperature generally produces a decrease in bulk and particle density, and there is a greater tendency for the particles to be hollow.

According to Chegini and Ghobadian (2005), the effect of temperature on bulk density depends on its effect on powder moisture content, as a product of higher moisture would tend to have a higher bulking weight caused by the presence of water, which is considerably denser than the dry solid. Particle density of the yoghurt powders varied from 1.38 to 1.44 g/cm^3. There were no significant changes in the particle density of the spray-dried powders for all the inlet temperatures. Data in the Table2 represents the averaged values of three determinations.

Dispersibility

There was no significant difference in powder dispersibility with increasing of inlet air temperature. dispersibility is affected by moisture content. The higher the powder moisture content, the more particles tend to stick together, leaving more interspaces between them and consequently resulting in decrease dispersibility. Triplicate samples were analysed.

Table 2. Physical properties of the spray-dried powder

Inlet temperature (◦C)	moisture content (%)	ρ_{bulk} (g/cm3)	$\rho_{particle}$ (g/cm3)	dispersibility (%)
150	4.59±0.01[a]	0.42±0.01[a]	1.44±0.04[a]	77.2±1.4[a]
170	4.09±0.5[a]	0.4±0.00[b]	1.38±0.00[a]	79.42±1.19[a]
190	2.8±0.16[b]	0.39±0.00[b]	1.38±0.09[a]	78.82±0.5[a]

Assays were performed in triplicate. Mean± SD values in the same column with different superscripts are significantly different ($p \leq 0.05$)

Color measurement

The results of the color measurement for powders are as shown in Table 3. L* is the lightness or darkness (L = 0, black; L = 100, white), +a is redness,.a is greenness, +b* is yellowness, and -b* is blueness. It was found that when inlet temperature increased, the +*b** values increased but the L values decreased. This implied that the color of the powders has become darker at higher inlet temperature. It is well known that high temperature can significantly decrease the color of yoghurt powder. A change in color of dried yoghurt powder is undesirable because it affects the consumer acceptance of the dried products. Triplicate samples were analysed and the mean was recorded.

Table 3. Colorimetric results of the spray-dried powders

Inlet temperature (◦C)	L	a*	b*
150	69.66±0.57[a]	0	7±1[a]
170	67.66±1.52[a,b]	0	8±1[a]
190	66±1[b]	0	9±1[a]

Assays were performed in triplicate. Mean± SD values in the same column with different superscripts are significantly different ($p \leq 0.05$)

Conclusions

Spray drying is a well-known process suitable for yoghurt drying because it allows preparation of stable and functional products. The results showed that inlet temperature has great influence on the physicochemical properties of the spray-dried powders. As inlet temperature increased, the moisture content, pulk density, particle density, L of the powder decreased while the dispersibility increased.

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Applications of flow cytometry and fluorescence techniques in somatic cell analyses of raw milk

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Monitoring the quality and safety of milk requires careful analysis of somatic cell count (SCC). The total of leukocytes and the cells secreted from udder tissues is composed somatic cells. At high level of SCC is a signal of diseases such as mastitis. It can be detected not only measuring the inflammatory components and pathogens but also evaluating the SCC in raw milk. Currently, enumeration of somatic cells relies on two methods such as direct microscopy and automated instruments in raw milk. Direct microscopy using trypan blue staining are time consuming method or it does not provide complete quality and safety assurance, or the instrumentation is limited in its range of application. The variety of the result of SCC due to the level of skills of operator is one of the drawbacks of this method. On the other hand, evaluating SCC with automated instrument such as flow cytometry is minimized the variety of results via its highly-sensitive property. Flow cytometry is a single instrument, rapid analyses of somatic and microbial cell count milk. This instrument is based on fluorescent stains or fluorogenic substrates in combination and the sample is pumped through a flow cell of very small diameter which allows only one cell to pass at a time. In order to count the somatic cell, the fluorescent dye penetrates the cell and contracted with nuclear DNA . In this study ethidium bromide (EtBr) and acridine orange (AO) were chosen to stain the somatic cells. EtBr is a large, flat basic molecule that resembles a DNA base pair because of intercalating into a DNA strand. AO binds to double-stranded nucleic acids fluoresces green. EtBr stock dye is advised to determine the SCC by AOAC method. AO is an alternative chemical dye for EtBr. Besides of these, while Etbr binds to dead cells, AO can binds both dead and live cells. The purpose of this study is to demonstrate the applicability of the flow cytometry using different dyes for detection of somatic cells in raw cow`s milk samples. Furthermore differences between flow cytometry and direct microscopy on SCC were also investigated in this study. Prior to SCC analyses, milk samples were centrifuged to remove the fat content. Cleared milk pellets were suspended in phosphate-buffered saline (PBS) and triton-X-100 solutions. After dilution, suspended somatic cells were stained with two fluorescent dyes including AO and EtBr. Stained samples were analyzed using flow cytometry (FACSCANTO BD, USA). These two dyes and their different applications were compared in terms of somatic cell counts and the obtained results were also compared with direct microscopy method. There is a good correlation between the somatic cell counts obtained through application of two fluorescent dyes (p> 0,05). The flow cytometry counts were also correlated strongly with the direct microscopy counts (p>0,05)

Effect of membrane clarification on chemical composition of mulberry juice

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Abstract

Mulberry juice was clarified with the membrane processing with mixed cellulose ester (MCE) membranes. Chemical properties of mulberry juice were measured to determine the effect of membrane processing on the juice properties. Also, the effect of membrane pore size (0.025, 0.1 and 0.22 μm) on juice composition was evaluated. Turbidity which was measured with portable turbidimeter decreased by 99.9% in all membranes. The pH was measured with digital pH meter and did not show any changes in all pore sizes; however, the acidity of samples, which was determined using titration with alkaline, decreased by 40.8%, 39.1%, and 36.8% in the MCE 0.025, 0.1, and 0.22 μm, respectively. Total soluble solids content was measured using a portable refractometer. Results showed that it was decreased by 40% in MCE 0.025 and by 26% in both MCE 0.1 and 0.22 μm. Total phenolic contents of the juice were measured using a Folin and Ciocalteau assay by means of a UV-Vis spectrophotometer. Juice treatment with MCE 0.22 μm has the less effect on the reduction of phenolic compounds (by 59.7%), but two other membranes approximately had same effect on the reduction of phenolic compounds (by about 74%). Total anthocyanin was measured with pH differential method and the results showed that MCE 0.025, 0.1 and 0.22 μm decreased it by 58.2%, 55.5%, and 43.8%, respectively. Antioxidant activity was measured based on the radical scavenging properties of the juice using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method which showed more reduction in MCE 0.025 μm compared to others.

Key words: Clarification, juice, red plum, membrane

Introduction

Black mulberry (*Morus nigra* L.) is one of the most nutritious fruits native to Iran. Black mulberry juice is useful in treating diseases such as anemia, pallor, dizziness, insomnia, and heart-palpitations (Pinelo *et al.*, 2009). Mulberry juice in its original state has a turbid appearance, which makes it difficult to process and preserve. Most industrially processed fruit-based beverages, including fruit juices and wines, are clarified as part of processing in order to avoid undesirable turbidity, haze, and sediments in the final products (Pinelo *et al.*, 2009).Current membrane-processing technology constitutes consolidated systems used in various productive sectors for their capacity to operate at room temperature and with low energy consumption (Rai *et al.*, 2005). Conventional clarification methods have been essentially replaced with membrane clarification, namely ultrafiltration (UF) and microfiltration (MF). UF and MF are two pressure-driven processes that can clarify large pieces from fruit juices but permit small solutes to pass through the membrane along with the water component (Rai *et al.*, 2005). In addition, membrane clarification can be used as a pre-treatment process to concentrate fruit juices using nanofiltration (NF) and reverse osmosis (RO).

Physic-chemical properties of fruit juice can change during membrane clarification. Matta *et al.* (2004) evaluated the effect of clarification of acerola juice after enzymatic treatment using polyethersulfone membrane with pore size of 0.3 μm on its physical-chemical characteristics. They observed that the pH, the viscosity, the pulp content, the proteins, the carbohydrates, the caloric value and the haze of the juice were decreased after membrane clarification. However, the soluble solid was constant and the acidity and the brightness were increased. In another study, Vaillant *et al.* (2005) considered the effect of clarification of enzymatic treated melon juice using ceramic membrane with pore size of 0.2 μm on its chemical

properties. They concluded that membrane processing can decrease some characteristics such as total soluble solid, viscosity, polyphenols. However acidity and carbohydrates were increased after clarification. Galaverna *et al.* (2008) clarified blood orange juice using PVDF membrane with nominal molecular weight cut-off 15 kDa and evaluated the effect of this process on chemical characteristics of the juice. They illustrated that the total antioxidant activity and two measured anthocyanins were constant after membrane treatment. However, ascorbic acid was decreased after clarification. Moreover, there are several similar studies which evaluated the effect of membrane clarification on physical-chemical characteristics of the juices. However, there is no study about membrane clarification of black mulberry juice. In current work, the effect of membrane clarification with mixed cellulose ester (MCE) membranes which have pore sizes of 0.025, 0.1 and 0.22 μm on juice composition was evaluated.

Material and methods

Juice extraction

Black mulberry (*Morus nigra* L.) variety Malaghi was prepared from fruit obtained at a local market (Karaj, Iran). The juice was extracted using manual pressing. To make the juice uniform for all experiments it was passed throughout a mesh filter (No. 10) before each experiment to remove very large particles.

Membrane units

Experiments were performed in a batch mode using a laboratory-scale plant (Fig. 1). The feed temperature was adjusted by circulating water in a two-layered tank. A rotary van pump (PROCON, Series 2, Milano, Italy) was used to introduce the feed above the membrane surface in a cross-flow mode. A transmitter (WIKA, type ECO-1, Klingenberg, Germany) coupled with an inverter (LS, model sv015ic5-1f, Korea) was used to maintain the feed pressure at the desired levels. The permeate was collected in a product tank. The retentate was recycled to the feed tank. Pressures in the feed and retentate sides were measured using two separate pressure meters (WIKA, model 213.53.063, Klingenberg, Germany). Mixed cellulose ester (MCE) flat membranes with pore sizes of 0.22 μm, 0.1 μm, and 0.025 μm (Milipore, USA) and a total effective filtration area of 0.0209 m^2 were used in this study.

Determination of physicochemical properties

The turbidity of the samples were measured using a portable turbidimeter (WTW, 350 IR., Weilheim, Germany) at 25°C after diluting (1:2) with distilled water. Titratable acidity was determined by titrating with sodium hydroxide (0.1 N) until it reached pH = 8.1 and it was expressed as g citric acid per 100 ml (Fadavi *et al.*, 2005). The pH was measured using a digital pH meter (Metrohm, Herisau, Switzerland). The total soluble solid (TSS) content of the feed and permeate samples were measured using a refractometer (ATAGO, HSR-500, Japan) at 25°C and expressed in °Brix.

Total phenolic contents of the juice were measured using a Folin and Ciocalteau assay by means of a UV-Vis spectrophotometer (CECIL, model 2502, Cambridge, England) at 760 nm, where 1.0 mL juice was mixed with 1.0 mL solution of hydrochloric acid (6.0 M) and 5.0 mL 75% methanol/water solution. After thoroughly shaking the above solution in a water bath at 90 °C for 2 hours and cooling it down to the room temperature, it was diluted to 10 mL. Then, 1.0 mL of the final solution was mixed with 5.0 mL Folin and Ciocalteau solution and diluted again using 15.0 mL sodium carbonate solution (7.0 g/100 mL). Final solution was then taken to 100.0 mL by using distilled water and its absorbance was measured by means of the UV-Vis spectrophotometer at 760 nm. The blank solution was also prepared in the same way but water was used instead of the juice. Gallic acid was used as the external standard. The total phenolic content was recorded as mg of gallic acid in 100 mL juice (Çam *et al.*, 2009).

Antioxidant activity was measured based on the radical scavenging properties of the juice using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the results were expressed as efficient concentration (EC

50). According to this method, 0.1 mL juice sample was mixed with 3.9 mL methanolic solution of DPPH (25 mg/L) and the absorbance of the new solution was measured using the UV-Vis spectrophotometer at 515 nm and it was considered as $[DPPH]_t$. Also, $[DPPH]_{t=0}$ was determined in the same way except that methanol was used instead of the juice in the above solution. The remaining DPPH (% $DPPH_{rem}$) was determined following the expression below:

$$\%DPPH_{rem} = \frac{[DPPH]_t}{[DPPH]_{t=0}}$$

The results were expressed as EC_{50} values, i.e. the concentrations of antioxidant required for the 50% scavenging of DPPH radicals. Also, the working curve was prepared by using 50, 100, 150, 200, 250 and 300 mg/100 mL of DPPH solutions (Çam *et al.*, 2009).

Total anthocyanin content (TAC) of PJ was measured according to Çam *et al.* (2009) by using the pH differential method. In this method, the pH of KCl solution (0.025 M) was adjusted to one using the HCl. Then, 3.6 cc of this adjusted solution was mixed by 0.4 cc juice and its absorbance was measured using a UV-Vis spectrophotometer (CECIL, model 2502, Cambridge, England) at 510 and 700 nm until reaching the steady state and they were named A_1 and A_2. Also the pH of sodium acetate solution (0.4 M) was adjusted to 4.5 using the acetic acid and this solution was passed a similar way as above one and its absorbance at 510 and 700 nm were named A_3 and A_4. Total absorbance (A) was calculated as

$$A = (A1 - A2) - (A3 - A4)$$

Total anthocyanin content (TAC) of PJ measured as

$$TAC = \frac{A \times MW \times DF \times 100}{MA}$$

where MW, DF and MA are predominant anthocyanin molecular weight, dilution factor and molar absorptivity of predominant anthocyanin, respectively.

Rejection factor

The rejection factor for each parameter was calculated as:

$$\%R = \frac{\varphi_F - \varphi_P}{\varphi_F} \times 100$$

where %R, F and P are the percentages of rejection for parameter φ, feed and permeate, respectively.

Statistical analysis

All experiments were repeated twice and data sets were subjected to analysis of variance (ANOVA) and the Duncan's multiple range test using SAS 9.1 software.

Results and discussion

Black mulberry juice was clarified using MCE membrane with pore sizes of 0.025, 0.1 and 0.22 μm at transmembrane pressure of 2 bars and velocity of 0.3 m/s to evaluate the effect of membrane clarification on chemical properties of juice. Results showed that the turbidity was greatly decreased in all membranes due to reduction of large particles which cannot pass from membrane pores (Table 1). It means that the membrane processing can be successfully used to clarify black mulberry juice. Measurement of the acidity showed that it was reduced in all treatments (Table 1). It can be attributed to blockage of some acidic components in cake layer formed on membrane surface. In contrast with acidity, the pH of samples did not change after membrane clarification. Also, blockage of components in cake layer can decrease juice

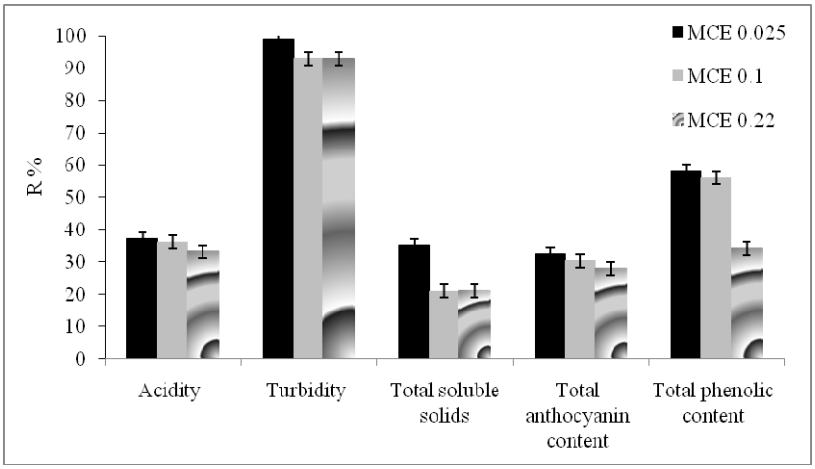
soluble solid content which can be seen in permeate chemical characteristics in all processes. Anthocyanins are sensible components to oxidation. According to this reason, total anthocyanin content in black mulberry juice decreased after membrane clarification (Table 1). Also, phenolic components decreased after membrane clarification due to same reason. Anthocyanins and phenolic component are two major components in black mulberry juice which have antioxidant activity. So, the antioxidant activity in black mulberry juice decreased after membrane clarification due to reduction of anthocyanins and phenolic components.

Table 1. Change in chemical compositions of black mulberry juice during membrane clarification

Characteristic	MCE 0.22 µm		MCE 0.1 µm		MCE 0.025 µm	
	Permeate	Feed	Permeate	Feed	Permeate	Feed
Turbidity (NTU)	16.5[f*]	12665[e]	13.3[d]	12874 [c]	11.7[b]	12349[a]
Total phenolic content (mg gallic acid/ 100 ml juice)	325.65[d]	[a] 809.16	209.58[c]	809.17[a]	206.63[b]	808.12[a]
pH	3.47[a]	3.45[a]	3.39[a]	3.45[a]	3.41[a]	3.45[a]
Total soluble solids (°Brix)	11[c]	15[a]	11[c]	15[a]	9[b]	15[a]
Acidity (g citric acid/100 ml juice)	1.1[d]	1.74[a]	1.06[c]	1.74[a]	1.03[b]	1.74[a]
Antioxidant activity (EC 50)	0.0064[e]	0.0023[a]	0.0068[d]	0.0021[c]	0.007[b]	0.0024[a]
Total anthocyanin content (mg 3-glucoside cyanine/ 100 ml juice)	9.4427[d]	16.8123[a]	7.4635[c]	16.7879[a]	7.0077[b]	16.7635[a]

* same letters show insignificant difference between values in each row (p>0.05).

Change value of each characteristic in all processes was measured to evaluate the effect of pore size on amount of change in chemical characteristics of mulberry juice. Results showed all membranes decreased turbidity by 99.9% and there is no difference between MCE 0.025 µm and other membranes in reduction of juice turbidity due to creation of cake layer on membrane surface in early stages of membrane processing which has dominant effect on reduction of juice turbidity. Also, the acidity decreased by 40.8%, 39.1%, and 36.8% in the MCE 0.025, 0.1, and 0.22 µm, respectively due to same result as turbidity reduction. Also, total anthocyanin content decreased by 58.2%, 55.5%, and 43.8% in the MCE 0.025, 0.1, and 0.22 µm, respectively due to same oxidation effect of process in all membranes (Figure 1). However, MCE 0.025 µm and MCE 0.1 µm reduced phenolic components more than MCE 0.22 due to large size of phenolic components which can retentate more with smaller pores. Also, MCE 0.025 µm decreased total soluble solids in juice more than other membranes.



Conclusion

Changes in chemical properties of black mulberry juice were evaluated during clarification with membrane processing. Results showed membrane clarification can decrease juice turbidity due to reduction of large particles which cannot pass from membrane pores. Same result was obtained for acidity which can be attributed to blockage of some acidic components in cake layer formed on membrane surface. There is no change in pH value of samples before and after membrane clarification. Also, soluble solid content of juices decreased after clarification due to blockage of components in cake layer and anthocyanins decreased due to oxidation. According to same reason, phenolic components decreased after membrane clarification. Antioxidant activity of black mulberry juice decreased due to reduction of anthocyanins and phenolic components as two major antioxidant components.

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Microstructure of Iranian processed cheese

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The effects of the concentration of trisodium citrate (TSC) emulsifying salt (0.25 to 2.75%) and holding time (0 to 20 min) on the textural, rheological, and microstructural properties of Iranian Processed Cheese Cheddar cheese were studied using a central composite rotatable design. The loss tangent parameter (from small amplitude oscillatory rheology), extent of flow (derived from the University of Wisconsin Meltprofiler), and melt area (from the Schreiber test) all indicated that the meltability of process cheese decreased with increased oncentration of TSC and that holding time led to a slight reduction in meltability. Hardness increased as the concentration of TSC increased. Fluorescence micrographs indicated that the size of fat droplets decreased with an increase in the concentration of TSC and with longer holding times. Acid-base titration curves indicated that the buffering peak at pH 4.8, which is due to residual colloidal calcium phosphate, decreased as the concentration of TSC increased. The soluble phosphate content increased as concentration of TSC increased. However, the insoluble Ca decreased with increasing concentration of TSC. The results of this study suggest that TSC chelated Ca from colloidal calcium phosphate and dispersed casein; the citrate-Ca complex remained trapped within the process cheese matrix. Increasing the concentration of TSC helped to improve fat emulsification and casein dispersion during cooking, both of which probably helped to reinforce the structure of process cheese.

Study of an antioxidant-rich blend marinade to minimize the formation of heterocyclic amines

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Heterocyclic amines (HAs) are mutagenic/carcinogenic substances formed in significant amounts during heating of meat and fish at household conditions.

As human diets include meat, it is impossible to avoid the risk of exposure to the heat generated contaminants. Minimization strategies of their formation are of great importance from the viewpoint of food safety, along with consumers' acceptance.

There are several factors involved in HAs inhibition, though it is apparent that antioxidant-containing juices/spices/herbs play a role in this process. This study proposed to understand the antioxidant-rich ingredients, including different marinade liquid with or without addition of a selected spices/herbs blend, in modulation of HAs.

Pilson beer and white wine were tested as marinade vehicles, the herbs/spices used were ginger, garlic, rosemary, thyme, red chili pepper. The marinating was performed during 4 hours before grilling the beef. Radical scavenging activity (RSA) was tested for all the marinades (B-beer; W- white wine; BH-beer and herbs; WH-wine and herbs) using DPPH assay. HAs were extracted by SPE and determined by HPLC-DAD/FLD.

Concerning RSA, marinades rank order was: W>WH>BH>B, with no significant correlations between HAs formation. Concerning marinades HAs inhibition the rank order was BH>B>WH>W. All marinades reduced total amount of HAs formation, the strongest effect was found for BH (92%). The phenolic profile of each marinade appears to be the key factor for HAs behavior.

These results suggest a good potential for practical application of this blend in daily cuisine, through the creation of an easy-to-use condiment with appropriate shelf-life and proven health benefits.

Texturing and swell-drying of chicken meat by instant controlled pressure drop DIC

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Dried meat in sliced, in cubic form or as powder, can be used as an ingredient for ready-to-eat noodles. Hot air drying is generally used, although it is well known that this technique leads to low quality, in terms of both physical and biochemical properties. The objective of the present work was to improve the traditional hot air drying of chicken meat using the instant controlled pressure drop DIC as texturing process in order to obtain a final high quality product. DIC technology is a High Temperature – Short Time treatment followed by an abrupt pressure drop (pressure-drop rate > 0.5 MPa s^{-1}) toward a vacuum (about 5 kMPa). DIC technology has been defined and developed since 1988 firstly as an expansion process perfectly adapted for heat sensitive foodstuffs, such as fruits, vegetables, milk and milk products.

In this study DIC was used as pre-treatment for texturing fresh chicken meat. RSM (Response Surface Methodology) was adopted with a saturated steam pressure ranged from 0.35 to 0.7 MPa during 82 to 138 s as holding time after 1 min cooking at 0.1 MPa. Drying kinetics of the control sample (cooked during 1 min at 0.1 MPa without dropping pressure) and DIC treated samples were studied in 40 °C/1 m s^{-1} hot air oven. Rehydration kinetics and holding capacity (WHC) were carried out at room temperature (23 °C). By modeling both drying and rehydration kinetics, effective diffusivity D_{eff}, starting accessibility W_s and final water content were performed. Analysis of apparent density carried out on various meat powders, allowed identifying the modification of structure issued from DIC treatment; scanning electron microscopy (SEM) was performed to detect microstructure modifications.

The obtained results showed significant impacts of DIC pressure on water holding capacity WHC and apparent density of powder. The higher the pressure, the higher the WHC and the lower the apparent density of powder. D_{eff} was improved by DIC treatment reflecting short time drying and rehydration. These results can be explained as due to the structural modifications resulting from DIC such as an open and porous texture increasing the space between the protein filaments as a result of an increase of the negative charges on these proteins.

This study will be completed by investigating biochemical modifications of proteins.

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